RELATIVE IMPORTANCE OF RECRUITMENT, SUBSTRATE AVAILABILITY AND ENVIRONMENTAL STRESS IN DETERMINING OYSTER RESTORATION TRAJECTORIES ACROSS AN ESTUARINE SALINITY GRADIENT

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ABSTRACT

Jonathan R. Lucas: Relative importance of recruitment, substrate availability, and environmental stress in determining oyster restoration trajectories across an estuarine salinity gradient
(Under the direction of F. Joel Fodrie)

Ecological processes in natural systems can bottleneck restoration attempts, yet ecological theory provides insights to overcome restoration challenges. I examined processes of environmental stress, recruitment limitation, and substrate limitation in the context of an oyster restoration network. Patch reefs on the restoration sites were either made of substrate that had been "seeded" with juvenile oysters to simulate a recruitment pulse, or unseeded substrate. Comparing oyster densities between seeded and unseeded substrate after 18 months demonstrated the extent to which the recruitment pulse determined population density. Population densities across the sites were strongly correlated to recruitment, indicating recruitment limitation affected the entire restoration network. Larval settlement was correlated with salinity, and sites farther upstream were decreasingly substrate limited. Mortality rates were density independent and likely related to stress from low salinity. Five of six sites exceeded criteria of restoration success, although continued monitoring will assess restoration trajectories over longer time scales.

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CHAPTER 1: RELATIVE IMPORTANCE OF RECRUITMENT, SUBSTRATE AVAILABILITY, AND ENVIRONMENTAL STRESS IN DETERMINING OYSTER RESTORATION TRAJECTORIES ACROSS AN ESTUARINE SALINITY GRADIENT

Introduction

Human society's inevitable reckoning with anthropogenic habitat loss continues to become unavoidable. While early conservationist thought focused on the preservation of existing habitats, conservation efforts have over time increasingly adopted restoration as a major tool. A net increase in habitat area and associated ecosystem services may be necessary, and accomplished by restoration of previously degraded systems (Turner 1994). Over the past 40 years, restoration ecology has accelerated as both a scientific discipline and a philosophy (Aber and Jordan 1985, Cairns 1993) to the point that the United Nations has named the years 2021 – 2030 "The Decade on Ecosystem Restoration."

In order for restoration to achieve the highest possible level of ecosystem recovery, practitioners must establish realistic goals, collaborate with a diverse group of stakeholders, and appropriately assess indicators of progress (Gann et al. 2019). Ecologists play an important role as educators and stakeholders in restoration projects. Further, restoration projects can double as experiments to test the robustness of fundamental ecological principles, and the application of this knowledge for the concurrent success of restoration efforts (Peterson and Lipcius 2003).

Ideally, when the causes of degradation have been removed or mitigated, the ecosystem will return by natural regeneration (Connell and Slatyer, 1977). However, in certain cases "bottlenecks" – factors or processes that constrain or prevent population growth – determine restoration progress (Gann et al. 2019). When attempts are made to return system parameters to their pre-degradation level, original biotic population and community structure may not necessarily comply, a phenomenon called hysteresis (Beisner et al. 2003, Johnson et al. 2017). This usually occurs because the system has stabilized in its alternate state (Lewontin 1969, Holling 1973). Significant efforts in active restoration of communities

and populations may be necessary to overcome restoration constraints. These efforts include assisted regeneration to help trigger natural recovery, up to full reconstruction of habitat and complete re-introduction of desirable biota (Gann et al. 2019).

A hospitable abiotic environment is necessary for restoration success, as environmental stresses may prevent the desired species from returning and establishing sustainable populations. For example, salt stress can prevent marsh grasses from recolonizing disturbed bare space (Bertness et al. 1992), and reduced atmospheric moisture following deforestation in the Amazon rain forest can inhibit reforestation (Staal et al. 2020). As abiotic environmental stress decreases, biotic factors begin to increase in importance in their effect on communities and populations (Menge and Sutherland, 1987).

Necessary to a discussion of the role of biotic factors is an understanding of how life-history traits influence population dynamics. Many plants and most non-mammalian marine species have a two-stage life history, beginning as a dispersive propagule (seed or larvae) followed by settlement on a site and metamorphosis into an adult form. In the marine environment, open populations in an area are connected by pools of larvae from which they are replenished by larval settlement. A group of spatially separated open populations that are connected by a common larval supply is considered a metapopulation (Kritzer and Sale 2004). Less commonly, some populations are not connected to others by larval dispersal. These are considered closed populations, and their new recruits can only come from the offspring of the parent population. While in a closed population the number of recruits will be dependent on the number of reproductive adults in the population, an open population will receive recruits independent of its own density (Caley et al. 1996).

Recruitment is the addition of individuals to the population, which in most marine populations occurs by the settlement of propagules. A population is recruitment limited if its population size during a relevant time period is determined by the number of propagules (settlers) that settle during that time period. (Hixon et al 2002). In a recruitment limited population, an increase in the number of settlers will definitively result in an increase in the population size, as is seen in many plant communities (Grubb 1977) including North American grasslands (Tilman 1997) and seagrasses (Orth et al. 2006).

Highs and lows in settlement directly impact population sizes in recruitment limited populations of the barnacle *Balanus glandula* (Gaines and Roughgarden, 1985), and the patchy supply of larvae has been shown to determine spatial distributions in population density of two species of damselfish on coral reefs (Doherty 1983).

If a population is not recruitment limited, it is most likely regulated by mortality from a density dependent, post-settlement process. Not all post settlement mortality occurs at density dependent rate, for instance mortality due to abiotic stress. However, in order for a population to be regulated at a stable level by post-settlement mortality, that mortality rate by definition must be density dependent (Caley 1996). Post-settlement processes that regulate population sizes and community structure are usually biotic in nature. For example, interspecific competition contributes to zonation of sessile invertebrate species in rocky intertidal environments (Connell 1961) and wetland areal coverage by native *Typha* spp. plants in the presence of invasive *Phragmites australis* (Paradis et al. 2014). Predation mortality can regulate biodiversity in marine populations (Paine, 1966) and occurs at a rate dependent of the density of the prey population, maintaining stability in that population (Hixon and Carr 1997). In such post-settlement regulated populations, population size will not be correlated to settlement magnitude (Gaines and Roughgarden 1985).

Density dependent population regulation is most important in populations experiencing high settlement, whereas populations with low recruitment tend to be limited by the number of recruits they receive (Gaines and Roughgarden 1985). However, many marine invertebrate population densities are influenced in some degree both by the magnitude of settlement events, and by density dependent mortality. There is not always a hard dichotomy (Chesson 1998). This dual scenario may occur in populations where density dependent mortality prevents a population from growing too large, but is not strong enough to completely erase the effect of a large pulse in recruitment (Caley et al. 1996).

Field assessment of recruitment limitation is difficult, as it is nearly impossible to count every settling larva. Researchers typically measure marine invertebrate recruitment as the number of new individuals that have been added to the population by settlement during a specific time frame. (Caley et

al. 1996, Roegner 1991). Undoubtedly, some settlers will die before the researcher has opportunity to count them. Thus, every measure of settlement in the field will miss certain amounts of early post-settlement mortality, and therefore underestimate actual settlement (Booth and Brosnan 1995).

Just as plant seeds require fertile soil, many marine invertebrate larvae must adhere to hard substrate for settlement and continued development. A population is substrate limited (or habitat limited in the case of site-specific reef fish, see Schmitt and Holbrook 2000) if its size is determined by the amount of available substrate. Substrate limited systems are similar to recruitment limited systems in that adult densities are correlated to settlement abundances, and not regulated by post-settlement mortality. However, in substrate limited systems the limiting factor is not the supply of larvae or propagules, but the supply of substrate (Schmitt and Holbrook 2000). Substrate availability is an important constraint to restoration work, for instance techniques such as "green gravel" have been recently developed to overcome substrate limitation in kelp forest restoration (Fredriksen et al. 2020).

Oyster reefs provide an excellent model system for applying and testing ecological principles in a restoration context. Oyster reefs are biogenic habitat which play a critical role in nutrient reduction, water quality, nursery and refugia for finfish and invertebrates, shoreline stabilization, and carbon cycling (Brumbaugh and Coen 2009, Grabowski et al. 2012, Fodrie et al. 2017). Despite these valuable ecosystem services, 85% percent of the earth's oyster reefs have been lost due to over-exploitation and habitat degradation (Beck et al. 2011). For example, the Eastern oyster *Crassostrea virginica* in the Chesapeake Bay is estimated to be only at 1% of its historical population level (Rothschild et al. 1994) and the situation is similarly dire on many other areas of the United States' Atlantic Coast (Zu Ermgassen et al. 2012).

Oyster reef restoration projects have been attempted on the Atlantic Coast of the United States for decades (Bersoza Hernández et al. 2018). Early oyster restoration projects focused on enhancing fishery production. During the 1990s, the ecosystem services provided by oysters gained public attention, and the goals of oyster restoration gradually began to shift from a fishery focus to an ecosystem focus (Brumbaugh and Coen 2009). Restoration success has been variable, and the scientific community does

not have a complete knowledge of factors causing success or failure. A 2007 analysis of oyster restoration in the greater Chesapeake Bay area concluded that restoration efforts up to that point had been misguided, that in the presence of disease and harvest historic ecosystem services in the estuary could not be returned, and that the fishery could only be sustained by aquaculture (Mann and Powell 2007). However, evidence emerged in 2009 in both the Chesapeake and Pamlico Sound, North Carolina, showing successful large scale oyster restoration in no-harvest sanctuaries (Schulte et al. 2009, Powers et al. 2009), although given the challenges inherent in oyster restoration, some of these declarations of success may be premature (Dunn et al 2014). The scientific community has continued to refine its corporate knowledge on restoration of oysters and their ecosystem services (Brumbaugh et al. 2006, Baggett et al. 2015, Fitzsimmons et al. 2020). Given the steep financial costs of restoring degraded systems compared to preventing their degradation (Bersoza Hernández et al. 2018, Bayraktarov et al. 2016) there is a demonstrable need for ecologically robust corporate knowledge to inform restoration and efficiently utilize resources (Gillies et al. 2017).

Ecological principles of population dynamics influence oyster restoration. Abiotic environmental stress is one barrier to oyster restoration. The Eastern oyster's physiological tolerances set its broad potential distribution across estuaries. For instance, the ideal salinity window for adult and juvenile oyster physiology is between 12-28 psu (Chanley 1958, Galtsoff 1964). Oysters can survive for weeks to months at 2 psu depending on water temperatures (Southworth et al. 2017), although gametogenesis will be halted below approximately 7 psu (Loosanoff 1953). Larvae require salinities above 10 psu for survival (Davis 1958). No oyster population can be successfully established and maintained in an area that the organism's physiology cannot tolerate.

Oyster populations can be recruitment limited (Brumbaugh and Coen, 2009, Fitzsimmons et al 2020) if the bottleneck to the population's growth occurs at or before the moment when motile pediveliger larvae settle and become sessile spat. Recruitment limitation is typically not found in areas that experience high oyster settlement (Geraldi et al. 2013). As is the case in most marine invertebrate populations, field measurements of oyster settlement will miss a certain amount of settler mortality.

Oyster larvae can be gregarious settlers, being drawn to substrate already holding spat and settling in a density dependent manner (Hidu, 1969). However, the density independent nature of oyster settler mortality allows for settlement to be inferred from recruitment. (Roegner 1991).

Oyster populations can be regulated by post-settlement factors. For example, Fodrie et al. (2014) found that the distribution of adult oysters on intertidal oyster reefs was uncoupled from the distribution of settlement, but rather was due to predation mortality in the highest settlement areas which produced higher adult densities in areas of low settlement.

Oyster populations may be substrate limited (Brumbaugh and Coen 2009, Fitzsimmons et al. 2020). Oyster shell is the preferred natural substrate for oyster larvae, and shell resources play a determining role in oyster population size due to oysters' reef forming behavior. Shell substrate is both a product of population growth and a habitat requirement for population growth. If shell substrate is not continually replenished, both recruitment, and population size will decline in a negative feedback loop as taphonomic processes continue to remove shell from the system (Mann and Powell 2007). Substrate availability can be as impactful to population size as are recruitment processes and density dependent mortality. Consequently, restoration attempts must be aware of the possibility of substrate limitation, and may require long term subsidies to shell/substrate budgets (Mann and Powell 2007).

An oyster population can be both recruitment limited and substrate limited (Brumbaugh and Coen 2009, Fitzsimmons et al. 2020) if 1) its population size is determined more by settlement than by density dependent post settlement mortality, and 2) addition of substrate would act to increase settlement and therefore the population. Oyster restoration practitioners may encounter scenarios where low recruitment is limiting the population, and a contributing factor to the low population is lack of available substrate to which potential recruits could adhere.

In this study, I examined restoration success and sources of limitation constraining oyster restoration success on six subtidal reef sites along a salinity gradient in a temperate, mesohaline estuary in North Carolina. To do so, I asked four specific, testable research questions.

1. **Does abiotic environmental stress preclude oyster populations from existing in the study location?** To answer this, I looked at historical and current observational data of salinity and dissolved oxygen (DO) trends, to characterize the prevalence of salinities below 10 psu and hypoxia. I also looked for correlations between salinity/DO metrics and rates of population decline on the restored sites.
2. **Given permissive environmental conditions, to what extent are populations on restoration sites recruitment limited, or regulated by density dependent post settlement mortality?** To arbitrate between these competing forms of population regulation, I (a) conducted a settlement string experiment in order to estimate the amount of settlement and availability of larvae. (b) The experimental units on restoration sites included substrate "seeded" with high densities of young oysters to simulate a recruitment pulse, alongside experimental units made of bare substrate. If the populations were recruitment limited (recruitment determines population size), at the end of the 18-month experiment I expected to see higher densities on the seeded treatment. If the populations were regulated by post-settlement density dependence, I expected the two treatments to have similar densities, as the effect of my simulated recruitment pulse would have been mitigated by post-settlement mortality. c) I examined correlations between population density on the same type of experimental units at the time of recruitment, and at a later period of time. If later density was correlated to recruitment density, recruitment limitation is indicated in the system. d) I tested correlation between the per capita rate of population change and oyster density. Positive correlation is evidence of post-settlement density dependence regulating the population.
3. **Are oyster populations on restoration sites substrate limited?** To test substrate limitation, I measured whether the addition of unseeded substrate a) resulted in an increase in population, and b) if that increase in population was sufficient of itself to meet metrics of restoration success.
4. **Do sites meet criteria of restoration success?** Powers et al. 2009 defined restoration success on restored reefs in Pamlico Sound, NC as oyster density > 10 oysters m^{-2}, presence of vertical reef structure > 10 cm, and evidence of recruitment in 1 out of 2 survey years. In the Chesapeake Bay, the

Oyster Metrics Workgroup (Allen et al. 2011) has defined restoration success as > 15 oysters m^{-2} (with a target of 50 oysters m^{-2}), stable reef height, and recruitment evidenced by two or more age classes. This working group also included oyster biomass and shell budget targets in their criteria, and designed their metric to be applied to oyster reefs six years after initial restoration. Referencing these previous metrics, I defined a reef site as successfully restored if, at the end of the experiment I saw (1) oyster densities > 15 m^{-2}, (2) presence of stable, vertical reef structure > 10 cm, and (3) evidence of recruitment in one out of two survey years.

Materials and methods

Study Area and Restoration Project

The New River Estuary is a shallow, turbid, eutrophic estuary in Onslow County, North Carolina (Hall et al. 2013). It drains a 1197 km^2 watershed which contains the City of Jacksonville (pop. 72,436 as of July 2019, www.census.gov) and Marine Corps Base Camp Lejeune, and empties into the Atlantic Ocean through a single narrow inlet, and well as into adjacent small sounds via dredged navigation channels (Figure 1).

The estuary is 25 km long, with a surface area of 88 km^2 (Crosswell et al. 2018). A large oyster population historically thrived in the lower 8 km of the estuary nearest the mouth (Winslow 1889). Oysters remain in that area, and since 2001 cultch plantings have been completed by the North Carolina Division of Marine Fisheries (NC DMF) throughout Stones Bay and into the lower reaches of Farnell Bay. Conditions in Stones Bay are sufficiently hospitable to oysters to allow it to currently be an active area for oyster mariculture.

Farnell Bay covers the portion of the estuary between 12 and 20 km from the estuary mouth. Oyster populations are not functionally present throughout the majority of Farnell Bay, and it is doubtful that they have been in recent history (Winslow 1889). The estuary floor consists almost entirely of sand and mud bottom, with very little hard substrate, excepting a NC DMF artificial reef AR 398, which was built in the upper reach of Farnell Bay in 2011 with the concrete rubble from a demolished nearby bridge.

A 2017 pre-project survey for the NREOH found some individual oysters living in parts of Farnell Bay on isolated pieces of substrate, indicating its abiotic conditions do not preclude oysters from living there.

Farnell Bay appears to be an ideal location to attempt to establish an oyster population. Healthy oyster populations exist very close by in adjacent Stones Bay. Pushing the population slightly farther up the estuary, thus into an area with presumably slightly lower salinities, would allow researchers to experiment on the boundary of a key environmental variable that controls many reef processes such as settlement and growth (Walles et al. 2016). This is particularly relevant in the context of global change, where saltwater intrusion (Bhattachan et al. 2018) and increased precipitation (Trenberth 2011) may be shifting the locations of suitable environmental envelopes for coastal foundation species (Tice-Lewis, 2018). Additionally, expanding the footprint of an ecosystem engineer like the oyster as far upstream as possible maximizes benefits from its ecosystem services for enhanced water quality and nutrient reduction.

In April of 2019, a restoration team led by the City of Jacksonville built six 0.21 hectare oyster reefs in Farnell Bay, with the objective of reducing nutrient levels, improving water quality, and providing reef habitat for fish and other estuarine organisms. The six reefs were designed to act as a series of habitat "stepping stones", which in addition to supporting improved water quality, could help to establish oyster brood stock incrementally up the estuary, including to AR 398. The restoration project was titled the New River Estuary Oyster Highway (NREOH) (Fig. 2).

While oyster shell has been considered the optimal substrate for oyster restoration, there is uncertainty as to its availability in quantities necessary for reef restoration and maintenance (Mann and Powell 2007). Manufactured substrate is also commonly used (Goelz et al. 2020), including Reef Ball™ and Oyster Castle®. Oyster Castle blocks, a cinderblock derivative, have shown an enhanced proportion of live recruits, recruit retention, and oyster biomass compared to unconsolidated shell in mesocosm and field experiments on intertidal oyster reefs in Virginia (Theuerkauf et al. 2015). For the NREOH reef construction, reefs were built of either Oyster Castle or Oyster Catcher™, a novel substrate from Sandbar Oyster Company made of cement infused into fiber bundles of jute erosion control cloth, and wet-formed

into a doughnut shaped “patty” approximately 5 cm tall and 27 cm in diameter (Fig. 3c). Oyster Catcher patties have higher rugosity and more interstitial space than cast concrete products such as Reef Ball or Oyster Castle, and biodegrade over time leaving the community of attached oysters.

Construction occurred in April 2019. Each reef site was positioned within 100 m of the shoreline, in water approximately 1m deep. One half of each reef site consisted of six rows of 15 patch reefs constructed from Oyster Catcher patties (hereafter “patties”) (Fig. 3a and 3c). These 90 patch reefs were built using 10 patties, each seeded with oysters that had settled during the previous year. Each patch reef consisted of two upper patties placed on eight lower patties which acted as a base (Fig. 3b). Seeded patties were installed to simulate a settlement pulse on experimental units which could be followed for the remainder of the experiment and to immediately establish a population to provide ecosystem services. The patties had been seeded remotely at intertidal aquaculture leases either in the Newport River approximately 63 km to the east, or in Bogue Sound 56 km to the east. At the seeding locations, the patties had been held on horizontal PVC poles, which rested on rebar racks in the lower half of the tidal range. Seeded patties were transported to the New River via barge, and were out of the water for approximately 24 hours during transportation. Deployment of the seeded patties created an immediate population of oysters on each reef site. In the middle of the 90 seeded patch reefs, I built four additional patch reefs using unseeded patties, to measure oyster recruitment to bare Oyster Catcher patch reefs. The other side of each reef site consisted of six rows of 15 patch reefs constructed from eight unseeded Oyster Castle blocks (hereafter “blocks”), also for a total of 90 patch reefs (Fig 3b and 3c). Each reef site thus had a total of 184 patch reefs within a 30 m by 70 m areal footprint (Figure 3a).

The seeded patch reefs served as the experimental treatment to examine recruitment limitation, and the unseeded patch reefs were the control. The patch reefs made of Oyster Castle blocks were not a perfect control for the treatment, as they consisted of different material. However, they allowed me an additional set of replicates to measure recruitment at reef sites, and to examine differences in oyster settlement preference between two fabricated substrate materials.

Sampling Methods

To approach my four research questions, I gathered data on salinity and dissolved oxygen at restoration sites (Question 1), estuary wide patterns of settlement (Question 2), recruitment localized to my restoration sites (Questions 2-4), and oyster population dynamics on the restoration sites (Questions 2-4).

To examine the effect of the New River's abiotic environment on recruitment and population dynamics, I measured salinity with a refractometer at settlement string locations every time I deployed or retrieved a string (see next paragraph for a description of the settlement string experiment). Using a refractometer, I measured salinity at each reef site approximately every 2-4 weeks throughout winter and spring of each year, and every 1-2 weeks during the summer and fall. This resulted in a total of 20-23 salinity measurements per site each year. From July 2 to October 28, 2020, I recorded salinities at sites 1, 4, 5, and 6 every two hours using HOBO U24-002-C conductivity loggers hung approximately 30 cm below the surface, gathering approximately 1300 point measurements of salinity at each of the four deployment sites. While I did not have enough loggers to deploy one at all six sites, the placement of these four loggers was designed to give an accurate picture of the distribution of salinities in Farnell Bay. I measured dissolved oxygen profiles at each of the reef sites on a biweekly basis from May to September 2020 using a YSI 6600 V2-4.

To better understand the patterns of settlement in the estuary, and particularly in Farnell Bay, I placed a series of settlement strings along the main axis of the estuary, beginning at 3 km from the inlet (12 km below my lowest restoration site) stretching up to 28 km from the inlet (8 km above my uppermost restoration site). To make each settlement string, a small hole was drilled through the center of 10 clean oyster valves, through which a galvanized steel wire was strung. I hung a series of strings along the center channel of the estuary, at intervals of approximately 1200m (Fig. 4). Each string was suspended approximately 50cm below the surface. I retrieved them approximately every two weeks from late May to mid-September in both 2019 and 2020, replacing each string with a fresh one upon retrieval. During retrieval of each string, I measured surface salinity using a refractometer. In the laboratory, I examined

each shell under a low power microscope, counting the total number of spat that had settled on the 10 shells during that period.

My counts are not an exact representation of settlement, as during the two-week deployment time it is possible that some of the settlers died. However, settler mortality is usually a density independent process, and therefore the number of surviving oysters I counted can be considered a proxy for settlement (Connell 1985, Roegner 1991).

On a monthly basis from June to October 2019, and July to October 2020, I sampled the density of oysters growing on the seeded patty patch reefs. I selected six patch reefs per reef site to be sampled throughout the experiment in 2019, and followed a different six patch reefs at each site throughout 2020. On each patch reef to be sampled, two patties were selected, one from the upper portion of the patch reef, one from the lower portion. A 100 cm^2 quadrat was placed on each patty three times, once haphazardly on the top, side, and bottom of the patty. I counted and measured all living oysters for which any part of their body fell inside the quadrat.

At each sampling episode, I sampled two of the four patch reefs made of unseeded patties. I selected patch reefs and patties so as to evenly sample each of the four patch reefs over the course of the summer. Quadrats counts used the same method for unseeded patch reefs as for the seeded patch reefs.

On both patty treatments, I scaled the density of oysters measured in the quadrats up to the number of oysters on the patty, using the rule that the top and bottom of a patty each consisted of a surface area of 575 cm^2 of substrate, and the patty side had a surface area of 425 cm^2 of substrate. This rule is based on standard patty being considered a cylinder with a 27 cm diameter and 5 cm height. If the specific patty was one of the upper two patties on that patch reef, I multiplied its number of recruits by 2. If that specific patty was one of the lower eight patties, I multiplied its number of recruits by 4. (I chose to scale my measurements of recruitment density to a six-patty patch reef in order to standardize comparisons between seeded and unseeded patch reefs, as will be discussed in the next paragraph.) Adding these two products together gave an estimate of the total number of oysters on that patch reef. I used recruits per patch reef as my standard metric of oyster density, as it is an easily visualizable quantity

and practically applicable to restoration practice. Most published studies record oyster densities in units of oysters per m^2 of seafloor (Powers et al. 2009, Theuerkauf et al. 2015). Each patch reef occupied approximately 0.3 m^2 of seafloor, interspersed with areas of bare seafloor in between them. Thus, any given square meter of reef sit floor contained only one patch reef, and the metric of oysters per patch reef therefore is comparable to oysters in a square meter of an individual reef site's areal coverage plus surrounding seafloor. In this thesis, I use oysters per patch reef as an appropriate proxy for oysters per m^2. I must also note that the total three-dimensional surface area of six Oyster Catcher™ patties is approximately 1 meter, so a patch reef is also an appropriate approximation of 1 square meter of three-dimensional substrate surface area on patty path reefs.

During my 2019 sampling, I observed that the lower portion of many of the seeded patch reefs had begun to subside, burying the lowest four patties under the seafloor sediment. By July 2020, this process had completely occurred on all patch reefs I sampled. When calculating oysters per patch reef, I chose to scale the oyster counts to a six-patty patch reef, not a ten-patty patch reef in order to exclude the buried patties. I did this for two reasons (1) because the vast majority of oysters on the lowest four patties were dead by the end of the experiment, thus making each patch reef functionally consist of only six patties. (2) By excluding these patties from my calculations from the beginning, I have removed burial as one of the causes of population decline, allowing me to focus on other variables. Sedimentation is indeed a variable that has negative effects on oyster viability (Fodrie et al. 2014, Colden and Lipcius 2015) and the relationship between bottom type and burial has been taken into account during the planning of other restoration projects (Theuerkauf and Lipcius 2016). However, I was not interested in studying the effect of burial because it was approximately constant at all sites, and did not answer any of my primary research questions. I suspect that burial was due to the mass of the individual patch reef as it settled with gravity, and the sediment that naturally accumulates on the up-current side of vertical structures. Thus, burial is to some extent likely an artifact of patch reef design that could be mitigated in future restoration work by engineering controls.

As a replicate unseeded treatment, I sampled accumulated oyster densities on unseeded Oyster Castle patch reefs (blocks) from June to October 2019, and once again in July, 2020. At each sampling event, I sampled four patch reefs. I sampled two blocks from each patch reef, one from the upper portion of the patch reef, and one on the lower (Figure 3b). All living oysters found growing anywhere on the chosen block were counted. Like the patch reefs made of bare Oyster Catcher™ patties, these patch reefs had been installed as bare substrate material when built in April 2019, therefore all oysters counted on each patch reef had recruited during the course of the experiment. To calculate oysters per Oyster Castle block patch reef, I multiplied the number of oysters counted on the upper block by 2, and the number of oysters counted on the lower block by 6. I calculated the mean number of recruits per patch reef each month at each site (n=4) and the standard error of the mean.

Analyses

To determine if my abiotic measurements were consistent with longer term trends in the estuary, I examined a dataset of 202 measurements of dissolved oxygen, and 214 measurements of salinity from the North Carolina Department of Environmental Quality (DEQ) taken monthly from 1999 to 2019 (https://www.waterqualitydata.us) at the mouth of French's Creek, which is adjacent to my Site 3. I examined summary statistics for DO and salinity data including mean, minimum, and interquartile range.

To characterize dissolved oxygen at each individual reef site at during my sampling in 2020, I used the minimum DO recorded in the YSI profile between 0.7 and 1.5 m depth. I used data in this depth range as the data most likely to correspond to the depth of my patch reefs (Buzzelli et al 2002). I then extracted the total number of hypoxic observations (< 2 mg/L), and calculated the proportion of hypoxic observations out of the total number of observations.

For refractometer point measurements, I calculated mean, minimum, and maximum salinity taken at the surface. For the four months of HOBO data, I calculated the above summary statistics, plus median salinity and the proportion of observations under 10 psu, which I use as a minimum for optimal oyster physical viability. I compared differences in mean salinity at each site, as measured by the HOBOs, using non-parametric bootstrapping.

I modeled 2019 and 2020 proportional population decline on the seeded patch reefs as a function of mean salinity as measured by the refractometer, modeling restoration sites as random effects with random intercepts. I estimated p-values and confidence intervals using parametric bootstrapping. I also looked for linear correlation between per capita mortality rate on the seeded patch reefs at each site and mean salinity as measured by the HOBO loggers, and of the proportion of HOBO measurements under 10 psu.

I looked for linear correlation between the mean density of oysters on both unseeded patty and block patch reefs at each site in October 2019 and mean refractometer salinities for the sites in 2019, to identify an effect of salinity on recruitment at the sites. In order to test the effect of salinity on mortality of the 2019 recruit cohort on the block patch reefs, I used linear regression to examine correlation between the salinity and proportional population decline from October 2019 to July 2020. For the salinity data I used seven refractometer measurements at each site taken approximately monthly from December 2019 to June 2020. To characterize population decline I used the change in a sites' mean oyster density between October 2019 and July 2020.

Based upon the number of spat counted on each string during the settlement string experiment, I calculated the mean number of spat per string for each of the 17 locations, during both 2019 and 2020. In 2019, I was unable to locate eight strings in the field, leading to a censored dataset. These eight missing strings came entirely from the lower portion of the estuary below Farnell Bay. Six of the eight lost strings had been deployed during late June and late September, when we noticed the highest settlement rates of the experiment. When not included in calculations of mean spat per string, these missing data inappropriately penalized spat counts in the lower estuary. Using the 'mice' library in the computer program R, I filled those eight values by multiple imputation via predictive mean matching. These imputations increased the mean spat for those locations, which better characterizes their actual settlement.

In both years, I plotted mean spat per string against most direct over-the-water distance from New River Inlet, and also against each location's observed salinity. The salinity value was the average of all bi-weekly salinity measurements for that location during the experiment that year. To examine correlation

between salinity and spat number, I extracted the mean spat per string values occurring in mean salinities between 11 and 28. I used this range of salinities because settlement dropped to zero below a mean salinity of 11, and also began to decline precipitously above a salinity of ~30. The decline in settlement at high mean salinity was only observed in 2019, as mean salinities in 2020 did not reach above 25. I modeled these data with analysis of co-variance (ANCOVA).

I compared mean spat per string at the five locations in Farnell Bay to the five locations in the lower portion of the estuary where oyster populations have historically thrived (Winslow, 1889). I did this separately for 2019 and 2020. In 2020, one of my locations in the lower estuary was unusable and my mean calculations only comprised the other four locations.

To characterize rates of population decline (a proxy for mortality) on the seeded patch reefs, I used a mixed effects linear model to model changes in population density at each site over time. Densities on each patch reef within the sites were modeled as random effects with a random slope and intercept. Models were fit using the "blme" library in R. I used parametric bootstrapping to estimate confidence intervals for the slope parameters at each site. Since my field sampling was split between two different years, and I sampled a different set of patch reefs on the sites each year, I modeled the 2019 data and 2020 data separately.

To have a measure of density on the seeded patch reefs at the end of the experiment to which I could compare densities on unseeded patch reefs, I calculated the mean number of oysters per patch reef (n=6) and the standard error of the mean at the final sampling event in October 2020. On the unseeded patty patch reefs, I also calculated the mean number of oysters per patch reef each month at each site (n=2). The unseeded patch reefs on the reef sites had remained in the water throughout the 18-month experiment, and thus all recruitment observed on these patch reefs at any given time during the experiment was cumulative. My monthly sample size (n=2) on the unseeded patch reefs was insufficient to be usefully modeled. At each site, I compared mean oyster densities on seeded patch reefs to mean oyster densities on unseeded patch reefs in October 2020 to determine if the seeding treatment had an effect on oyster density. I made the comparison between seeded patch reefs and unseeded patty patch

reefs using non-parametric bootstrapping due to unequal sample sizes. I plotted the density differences between the seeded patties and unseeded blocks for visual comparison, but did not calculate statistical differences due to the dissimilarity of the two substrates. If oyster populations in Farnell Bay were recruitment limited, at the end of the 18 month experiment I would expect to see higher densities on the seeded patch reefs, as the recruitment pulse treatment would be the primary driver of population size.

I compared population density early in the experiment against population density on the same patch reefs later in the experiment, to determine the effect of recruitment on eventual population density. On seeded patch reefs, I compared densities in June 2019 to densities in October 2020. On unseeded patty patch reefs, I compared density as of October 2019 (when I quantified the 2019 cohort) to density at the end of the experiment in October 2020. For unseeded block patch reefs, I compared densities in October 2019 to their densities in July 2020.

I explored whether the per capita mortality rate on seeded patch reefs was related to population density, to investigate effects of density dependent processes. To calculate per capita mortality I used the mean density of oysters measured on a site at a monthly sampling event, as a percentage of the mean oyster density measured the previous month, divided by the number of days between the two sampling events. I then separately modeled per capita mortality as a function of per patch reef oyster density that month using ANCOVA.

To test substrate limitation, my oyster density measurements on the unseeded patch reefs indicated whether or not adding bare substrate to Farnell Bay increased its oyster population. While the settlement and survival of one oyster on the bare substrate is a population increase, this increase is so small it is meaningless. I defined a meaningful increase as bringing oyster densities on bare substrate above success criteria of 15 oysters m^{-2}. For both unseeded patty and block patch reefs, I calculated mean oyster density and standard error of the mean. I plotted whether the addition of bare substrate increased the oyster population on that substrate above either my minimum metric of restoration success of 15 oysters m^{-2}, or my target metric of 50 oysters m^{-2}.

A comparison of patty and block substrate at the patch reef level has inherent limitations. An Oyster Castle block has a surface area of 5600 cm^2, which is over three times more surface area than a 1575 cm^2 Oyster Catcher patty. Additionally, the density calculations for a block patch reef were based on eight blocks, while the density calculations for a patty patch reef were based on six patties. To mitigate these differences, I compared densities between the unseeded patty and block patch reefs over 0.1 m^2 of substrate surface area (not seafloor) in October 2019 and July 2020, using means and standard error. The purpose of this comparison was to determine effects of larval preference for a particular substrate, and the effects of substrate material on post-settlement mortality in the context of substrate limitation.

Results

Abiotic Factors

The long term DEQ dataset shows monthly dissolved oxygen measurements to have a mean of 8.2 mg/L, with no measurement in the 20-year dataset dropping into hypoxia (< 2mg/L). Mean dissolved oxygen levels at each site from May to September 2020 varied from 7.1 to 7.7 mg/L (n=10). The lowest dissolved oxygen recorded at any site during this time period was 5.65 mg/L, which occurred on August 12, 2020 at Site 4. These measurements are well above a hypoxia threshold of 2.0 mg/L (Keppel et al. 2016).

Long term DEQ salinity data had a mean of 16.05 ppt, and an interquartile range of 12.24 to 20.10 ppt. 36 of the 214 measurements, or approximately 17%, were under 10 ppt. Biweekly salinity point measurements from the refractometer showed Site 4 to have the highest mean salinity in both years. Site 1 had the lowest mean salinity in 2019, while Site 6 had the lowest mean salinity in 2020. Mean salinities among Sites 2,3, and 5 were very similar in 2020. The spread in mean salinity among the sites was 3.4 psu in 2019 and 2.8 psu in 2020. In 2019 mean salinities were 8-9 psu higher at all sites compared to 2020 (Figure 5 and Table 1). Site rankings based on bi-hourly HOBO salinity measurements in 2020 were consistent with the two-year point measurements. From July 2 to October 28, 2020, Site 4 had the highest mean salinity (15.88) and the smallest percentage of observations under 10 psu (3%). Site 6 had the lowest mean (12.46) and the largest percentage of observations under 10 psu (15%). Mean

salinities at all four sites were statistically distinguishable from each other. Standard deviation of recorded salinities for all sites was between 2.29 and 2.51 (Figure 6 and Table 2).

These continuous data allow greater temporal resolution in salinity differences compared to point measurements. By mid-June 2020, the New River Estuary had entered a major freshet. Refractometer measurements on June 18 were between 0-3 psu at all sites. Continuous logging began on July 2, and showed that site 6 reached 10 psu on July 12, and 12 psu on July 21. Site 4 emerged above suboptimal oyster salinities weeks sooner, reaching 10 psu on July 3, and 12 psu on July 6.

I found some correlation between the 2019 mean salinity for each site and that site's proportional population decline on seeded patch reefs during the 2019 sampling season ($p = 0.054$) showing an 8% decline in population density for every psu (95% confidence intervals between 2% and 15%) (Fig. 7a). In 2020, I found no correlation between mean salinity and proportional population decline ($p = 0.538$, 95% confidence intervals overlap zero) (Fig. 7b). At Sites 1,4,5, and 6, using the HOBO measurements from 2020 I found no linear correlation between per capita mortality rate and mean salinity ($p = 0.89$) or proportion of salinity measurements < 10 psu ($p = 0.44$).

I did not detect an effect from salinity on proportional decline in mean density of the 2019 cohort on block patch reefs at each site ($p=0.81$) between October 2019 and June 2020. During this time period, the mean density on the unseeded block patch reefs at Sites 3 and 4 declined approximately 25%, while declining between 60-80% at Sites 1, 2 ,5, and 6.

I did not find strong statistical evidence that the mean density of the 2019 recruitment cohort on unseeded patties at each site was a function of the site's mean salinity for 2019 ($p = 0.16$, $R^2 = 0.28$) (Figure 8a). Similarly, I did not detect correlation between the density of the 2019 cohort on blocks and a site's mean salinity ($p=0.577$, $R^2 = 0$) (Fig. 8b). I found oysters in the 2020 recruitment cohort only at Site 1, and thus could not conduct inference on the effect of salinity among sites for the 2020 cohort.

Recruitment limitation

In 2019, mean spat density on settlement strings increased down the estuary toward the inlet, reaching a maximum of 11.7 spat per string at 10km from the inlet, which corresponded to a mean salinity of 30. Below this point, the mean number of spat per string began to decrease. (Fig. 9a and 9c).

In both 2019 and 2020, mean salinity per string location increased with proximity to New River Inlet. Overall salinity and settlement trends in 2020 were lower than in 2019. In 2020, I measured no settlement above 16km from the inlet (approximately halfway up Farnell Bay) (Fig 9b). Below this, spat per string increased as distance from the inlet decreased and salinity increased, reaching a maximum of 3.25 spat per string at my lowest location in the estuary. In 2020, the highest seasonal mean salinity for any string location was 24 (Figure 9d). Settlement occurred past 25 km upstream in 2019, but ended 17 km upstream in 2020. Farnell Bay covers the area of the estuary between 13 and 19 km from the inlet, so its position in the salinity settlement window changed from 2019 to 2020. Sites 1, 2, and 6 are > 17 km from the estuary mouth, while sites 3,4, and 5 are < 17 km from the estuary mouth.

A location's mean salinity explained 61% of the variance in mean spat per shell string (ANCOVA). In the range of salinities 11 – 28, the effect of mean salinity on mean spat abundance was the same in 2019 and 2020 ($p=0.23$), with a constant effect size of 0.26 spat per psu ($p < 0.001$ in 2019, $p = 0.007$ in 2020). This relationship held even as baseline spat densities were much higher in 2020, and the geographic position of this salinity window in the estuary shifted 8 km between years (Figure 9e).

In 2019, the mean spat per string for the combined five settlement string locations in Farnell Bay was 2.68. The mean spat per string for the combined five locations in the oyster's historic range in the lower New River (Winslow 1889) was 4.7. In 2020 in the lower estuary, the mean spat per string was 1.8, while mean spat per string in Farnell Bay was 0.2. Thus, I measured 93% less spat in Farnell Bay on settlement strings in 2020 than in 2019. Using spat per string as a proxy for settlement, settlement in Farnell Bay was 42% lower than it was in the lower estuary in 2019, and 90% lower in 2020.

The reef sites did not begin the experiment with similar mean oyster densities, which was an artifact of reef construction (Figure 10). Initial mean site densities were Site 4: 2075 oysters m^{-2}, Site 3:

1816 oysters m^{-2}, Site 5: 1027 oysters m^{-2}, Site 2: 732 oysters m^{-2}, Site 6: 731 oysters m^{-2}, and Site 1: 648 oysters m^{-2}. Oyster densities on seeded patch reefs at all sites clearly declined in 2019. The rates of decline at Sites 3 and 4 in 2019 were noticeably higher than those at Sites 1,2,5, and 6. Overall, populations declined much less in 2020 compared to 2019. Confidence intervals at Site 1 and Site 6 indicate that in 2020, rates of population decline were not clearly different than zero. These populations may have stabilized. In 2020, densities at sites 2, 3, and 5 declined at rates not clearly distinguishable from each other. These populations may be near to reaching stability. The rate of decline at Site 4 was clearly higher than at all the other sites (Figure 11).

By October 2019, the mean density at Site 1 had declined by 87% from its initial density as measured in June 2019. By the end of the experiment in October 2020, Site 1 had declined by 96% from its initial density. During the same time windows, Site 2 declined 79% and ultimately by 85%, Site 3 declined by 62% and ultimately by 84%, Site 4 decline by 65% and ultimately by 86%, Site 5 declined by 75% and ultimately by 90%, and Site 6 declined by 96% and ultimately by 98%.

Recruitment to unseeded patch reefs occurred at all sites throughout the summer of 2019, although it was much higher at sites 3,4,5, and 6. All sites experienced mortality of the 2019 cohort between October 2019 and July 2020. Despite demonstrating noticeable recruitment during 2019, by the end of 2020 Site 6's unseeded patch reefs held the lowest remaining mean oysters of all the sites (Figure 15). The margins of error that I calculated for the sample mean on unseeded patties at each site are broad due to small sample size (n=2).

In October 2019, 80.7% of recruits on bare patty substrate measured between 1-3 cm, 16.5% measured 4 cm, and 2.7% were 5 cm. Similarly, 83.4% of recruits on block substrate measured between 1-3 cm, and 13% measured 4 cm. This justifies a conservative use of 1-3 cm as a size to age proxy for Farnell Bay oysters in their first four months of life. When analyzing the October 2020 data from the unseeded patch reefs to estimate the size of the 2020 recruit cohort, I made the conservative assumption that any oyster 3 cm or less in length recruited to the substrate during 2020. I considered oysters 4cm or longer to have recruited during 2019. Patch reef level extrapolations based on my October 2020 data

show no oysters 3 cm or less on unseeded patty patch reefs at Sites 2, 3, 4,5, and 6. Unseeded patty patch reefs at site 1 had a a very low mean of 4.25 oysters ≤ 3 cm per patch reef, based on sampling one 2 cm oyster on one patch reef. These low densities on useeded patties indicate that across all sites, recruitment was extremely sparse in 2020. In addition, the 2019 recruit class experienced 82-100% decrease on unseeded patch reefs during 2020.

As of October 2020, the magnitude of difference between densities on the seeded and unseeded treatments varied by site. A difference indicates recruitment limitation. Descriptions below are listed in upstream to downstream order, as depicted in Figure 12a.

Site 1. I saw a clear statistical difference (p = 0.018), where mean densities on seeded patties (24.8 +/- 3.2) met minimum success criteria, and densities on unseeded patties (4.25 +/- 4.25) did not.

Site 6. Mean densities on seeded patch reefs (14.2 +/-4) may not meet minimum success metrics, and I was unable to detect a statistically clear difference (p = 0.269) between mean densities on seeded (14.2 +/- 4) and unseeded patch reefs (5.75 +/- 5.75).

Site 2. Mean oyster densities on seeded patch reefs (109.2 +/- 13) finished solidly above target success criteria, however October's sampling found no oysters growing on unseeded patch reefs. The statistical difference between seeded and unseeded patch reefs was clear (p = 0.009).

Site 3. Mean oyster densities on seeded patch reefs (286.3 +/- 38) exceeded target success criteria and were statistically distinguishable (p = 0.014) from densities on unseeded patch reefs (11.5 +/- 11.5).

Site 5. The spread of the data for Site 5 was large, and I was unable to detect a clear statistical difference between mean oyster densities on the seeded (99.8 +/- 32) and unseeded (30 +/- 21.5) patch reefs (p = 0.220). The fact that the mean on seeded patch reefs is 3x higher than on unseeded still suggests the possibility that the two treatments are different, although the trajectory of the two treatments may indicate a future confluence.

Site 4. Mean oyster densities on seeded patch reefs (280.6 +/- 28) exceeded target success criteria and were statistically distinguishable (p = 0.010) from mean densities on unseeded patch reefs (22.75 +/- 11.25).

Although I did not conduct a statistical comparison, as of July 2020 densities on seeded patch reefs were clearly higher than densities on unseeded block patch reefs (Fig. 12b). Differences were the highest at sites 2-5. The differences varied in magnitude by site, however some variation in magnitude may be due to the seeding densities on the patty patch reefs (Fig. 10).

On seeded patties, site level mean oyster densities at the end of the experiment were linearly correlated with site level mean oyster densities at the at the beginning of the experiment ($p = 0.003$, $R^2 = 0.89$). None of the sites had excessive influence on the linear fit (Figure 13a). On unseeded patties, oyster densities in October 2020 were linearly correlated with the estimated size of the 2019 recruit cohort ($p < 0.001$, $R^2 = 0.94$) (Figure 13b). On unseeded patties, the mean oyster densities for Site 5 had an outsized influence on the model fit (Cook's distance = 3.08). However, the linear relationship was still statistically clear even when Site 5 was removed from the data ($p = 0.025$, $R^2 = 0.81$) Densities on unseeded block patch reefs in October 2019 were correlated with densities in July 2020 ($p = 0.020$, $R^2 = 0.72$) (Figure 13c). None of the sites had an excessive influence on the model fit (Cook's distance < 1). Evidence of correlation on blocks is less reliable due to the clustering of points near the origin.

On seeded patch reefs in 2019, monthly per capita rates of population decline clearly decreased with increasing oyster density ($p = 0.006$), providing evidence of negative density independent mortality in that year. On seeded patch reefs in 2020, monthly per capita rates of population decline showed no correlation to oyster density ($p=0.88$) (Figure 14). Both of these results suggest that oyster mortality during the experiment was not positively density dependent.

Substrate limitation

At the end of sampling, oyster densities on unseeded patty and block patch reefs at upstream Sites 1, 6, and 2 fell below minimum success criteria, indicating that these sites were not substrate limited during the timeframe of the experiment (Fig 12). At downstream sites, densities clearly exceeded success criteria on unseeded block patch reefs at sites 3 and 5, indicating substrate limitation. The margin of error at site 4 overlapped minimum success criteria, keeping open the possibility of substrate limitation while not allowing a clear determination. Margins of error for the downstream unseeded patty patch reefs were

larger, overlapping minimum success criteria at sites 3, 5, and 4 (Fig 12), and again making substrate limitation on these patch reefs possible but not clear. Site 5 seemed to maintain the highest densities on both types of unseeded substrate.

When oyster densities were compared over an equivalent amount of individual substrate surface area, a difference between the two materials emerged (Fig 15). Using a scale of oysters per 0.1 m^2 of substrate material, the 2019 cohort recruited in higher densities to patty substrate than to block substrate (Fig 15). At upstream sites 1 and 2, mean recruitment of the 2019 cohort was 2 – 3 times higher on patties. Site 6 experienced remarkably overall higher recruitment of the 2019 cohort than did sites 1 and 2, which in turn was 26 times higher on patties than on blocks. Nine months later in July 2020, much of the differences between the substrates had been erased by post-settlement mortality. There was no practical difference in mean oyster density at sites 1 and 2 (densities were perhaps a little higher on blocks), and at site 6 the mean density on patties was 2.5 times higher on patties than on blocks (Table 3). At downstream sites 3, 5, and 4, recruitment of the 2019 cohort was 6-11 times higher on patty substrate material, as measured in Oct 2019. At site 3, this difference decreased during the subsequent nine months. While densities overall decreased during the winter and spring of 2020, at sites 4 and 5 the differences between the two substrates increased so that densities on patties were 12-16 times higher on patties than on blocks by July 2020 (Table 3).

Discussion

Restoration work in coastal biogenic habitat such as oyster reefs must account for multiple layers of factors influencing population dynamics. 18 months after restoration, I saw the best indications of restoration success at higher salinity, down-estuary sites. My results suggest that – within the optimal salinity window – settlement was correlated with salinity, and oyster populations in Farnell Bay were recruitment limited. Upstream sites of lower salinity were not substrate limited, while evidence of substrate limitation was stronger at downstream sites. It also appeared that density independent post-settlement mortality played an important role in depressing oyster densities, particularly at upstream sites,

and potentially from low salinity stress. My conclusions are limited to the time period 18 months post-restoration and could change over longer, ecologically meaningful time scales.

Even though the settlement string experiment suggests that settlement was correlated to salinity, I was unable to find significant correlation between salinity and 2019 recruit class density on unseeded patch reefs. If salinity does have an effect on recruitment, a possible mechanism for the effect of salinity on settlement is increased densities of larvae brought upstream by a salt wedge during flood tides. (Carriker 1951, Andrews 1983).

Settlement on settlement strings occurred in a consistent salinity window even while shifting between geographic regions of the estuary. The downstream shift in salinity window was most likely due to increased upstream freshwater discharge into the estuary. Monthly mean discharge rates measured at the USGS Gum Branch gaging station are an accurate indicator of freshwater input to the New River estuary (Hall et al. 2013). The mean discharge rate was 12 times higher in May 2020 compared to May 2019, and 35 times higher in June 2020 compared to June 2019 (https://waterdata.usgs.gov). My settlement string data showed highest settlement consistently in June during both 2019 and 2020, which is consistent with the time of peak oyster spawning in other areas of North Carolina (Puckett et al. 2014). The June 2020 New River freshet may have reduced settlement by increasing larval mortality in the plankton (Davis 1958, Dekshenieks et al. 1993), or the significant increase in estuary flushing from high freshwater discharge (Hall et al. 2013) may have prevented downstream larvae sources from connecting with Farnell Bay. Regardless of mechanism, the 2020 salinity shift largely excluded Farnell Bay from the area of optimum settlement, particularly above Site 3. While settlement can occur in Farnell Bay, I found it to be inconsistent year to year and even site to site.

My data suggest that lower salinities were associated with higher mortality rates on seeded patty patch reefs during 2019, while not influencing mortality rates in 2020. Since salinities in 2019 were generally optimal for oyster physiology, this increase on mortality may be driven by an unknown upstream covariate, and not low salinity directly. The oysters on seeded patties were transplanted from areas with large oyster populations and presumably high genetic diversity. One speculation is that some

transplanted genotypes were less able to cope with the rapid transition to a different environment or salinity regime, and experienced higher mortalities during the first year. By 2020, New River-compatible genotypes had been selected in the transplanted population, mortality rates decreased, and so did its correlation with salinity.

Freshets are common in Farnell Bay. Besides June 2020, there was a brief freshet in September 2019 following Hurricane Dorian, and prior to the experiment in September 2018 following Hurricane Florence (personal observations). Oyster restoration work in estuaries cannot ignore the system's salinity regime (Bagget et al. 2015, Walles et al. 2016). In a warming climate, an increase in precipitation can mean an increase in episodic freshwater stress to estuarine populations, despite longer term salinity averages that still appear favorable to oysters. Episodic freshwater stress can be especially impactful if concurrent with high temperatures. (Southworth and Mann 2017). Conversely, in some cases freshwater input may actually decrease oyster mortality by removing pests, provided it is of appropriate magnitude and duration (Livingston et al. 2000), particularly in subtidal reefs which otherwise remain perpetually available to oyster pests (Walles et al. 2016).

In discussing the relative importance of recruitment limitation, I have made the key assumption that each of my six restoration sites are part of an open population, in that they receive larvae via the plankton from each other and from other populations in the New River with which they are connected. While this study did not include measurements of larval dispersal, I made this assumption because (1) for most marine species, dispersal distances within closed metapopulations are on the scale of ten to one hundred kilometers (Cowen et al. 2006) while my sites are 2-3 km apart, and less than 10km from a known larval source in Stones Bay, (2) dispersion distances for oyster larvae in the nearby Pamlico Sound are on the scale of 5-40 kilometers (Puckett et al. 2014), (3) each individual site of 0.21 hectares is too small to be self-seeding, given typical larval dispersal times (Puckett et al. 2014), and (4) despite general downstream river flow, tidal currents in Stones Bay measured in 2009 indicate that on a flood tide, conditions are permissible for potential larval transport of up to 6 km from Stones Bay into Farnell Bay (Whipple et al. 2018 and Anthony Whipple, personal communication). While an open population means

that sub-populations receive larvae from the same pool, it does not mean that all sub-populations receive larvae in the same abundance, as physical and biological factors turn on and off different larval sources and sinks at different periods of time (Mann and Powell 2007).

Population densities on the seeded patch reefs in 2020 were linearly correlated to the size of the simulated recruitment pulse at the beginning of the experiment. Population densities on unseeded patch reefs in 2020 were linearly correlated to the abundance of the 2019 recruitment cohort. These tests indicate recruitment limitation. I did not find evidence that per capita population decline increased on seeded patch reefs with higher oyster densities, which suggests that mortality on seeded patch reefs is not density dependent. This result is surprising. The oysters on the seeded patch reefs demonstrated continual growth throughout the experiment, with the mode length doubling from 3 cm to 6 cm (Fig. 16) as oyster sizes increased. These seeded patties began the experiment with high oyster densities (Fig. 10) and it is logical to assume that competition for space would ensue as the oysters grew. Intraspecific competition in site-specific marine species typically results in density-dependent mortality (Hixon and Jones 2005, Webster 2004). Yet my data show no evidence of density dependent mortality, even suggesting negative density dependent mortality in 2019. Perhaps density thresholds necessary for competition induced mortality are higher for oysters than densities present on the restoration sites, or perhaps density independent mortality occurred at a rapid enough rate throughout the experiment that as oysters grew, there was sufficient space for all survivors. When taken as a whole, the linear correlation between recruit classes and adult densities, and the lack of density dependent mortality point toward recruitment limitation.

The comparison of densities between seeded and unseeded patch reefs yielded consistent site-by-site results, whether the comparison was made using patty or block patch reefs (Fig. 13) and was the most pronounced at sites 3 and 4. This is good evidence that sites 3 and 4 are strongly recruitment limited. The difference between seeded and unseeded treatments was clear at site 2, although not as pronounced as at the more downstream sites 3 and 4. The mean density on seeded patch reefs at Site 5 was similar to the mean at site 2, however site 5 showed much more recruitment, and therefore less of a difference between

the two treatments at that site. Densities on both treatments at the two most upstream sites (sites 1 and 6) were both similar and both very low. At Site 1 the density on the seeded patch reefs was barely above success criteria, but was statistically different than the unseeded patty treatment, so there is evidence of a small amount of recruitment limitation at site 1. At site 6 there was no difference between the seeded and unseeded treatment, indicating no recruitment limitation. These results together suggest a trend of decreasing recruitment limitation up the estuary, and are consistent with the results of the settlement string experiment, which indicated higher settlement potential in the downstream portion of Farnell Bay.

Geraldi et al. (2013) conducted a similar experiment, found no difference in oyster densities between seeded and unseeded substrate after 1-2 years, and reported no evidence of recruitment limitation. In their study, the two substrates reached parity because of high recruitment to the unseeded substrate, which reached oyster densities over 1000 oysters m^{-2}. This occurred while seeded substrate plots maintained their already-high densities. In my study, the sites which showed the greatest parity between treatments after 18 months (sites 1 and 6) did so not because of high recruitment to the bare substrate, but because steep mortality on the seeded substrate depressed those densities to the point that they were no different than the low densities on the low-recruitment unseeded substrate.

This reveals two very different scenarios where recruitment may not be limiting. In the first scenario, recruitment limitation is not found because as settler densities continue to increase, the influence of settlement on population dynamics decreases, and density-dependent post-settlement processes come to regulate the population (Hixon et al. 2002). In the second scenario, density independent mortality – apparently from environmental stress – is sufficiently strong to depress both settlement and post-settlement survival to the point that recruitment is too small to effect a signal in the population size, and post-settlement density independence cannot occur because the population never reaches the necessary density for it to be relevant. The first scenario happens when population densities are very high, the second when population densities are very low.

Differentials in survival based on oyster size may limit the accuracy of a comparison between oyster densities on seeded and unseeded patch reefs. Oysters best resist crushing by the blue crab

Callinectes sapidus upon growing to a shell length of approximately 30 mm (Eggleston 1990). Brown and Haight (1992) report that predation mortality from stone crabs *Mennipe adina* was higher in oysters weighing less than 20 g compared to oysters weighing 25-35 g or greater than 50 g, presumably due to increased predator handling time for heavier oysters. A shell height of 35-55 mm may provide a refuge from predation by invasive green crabs *Carcinus maenus* in Prince Edward Island, Canada (Pickering et al 2017). However, Booth et al. (2018) found no size refuge for oysters from predation by the crown conch *Melongena corona* in northeastern Florida. Although my results indicate predation was not a significant driver of mortality on the seeded patch reefs due to density independence, I did not investigate monthly mortality rates on the unseeded substrate, and so cannot rule out the possibility that 2019 recruit class suffered higher mortality on unseeded patch reefs throughout 2019 than did the recruitment pulse treatment on the seeded patch reefs. Yet mortality on seeded patch reefs was correlated with salinity, and it appears that there is little to no difference in tolerances to low salinity between spat and adult oysters (Loosanoff 1953, Chanley 1958). If mortality on both seeded and unseeded patch reefs is driven primarily by environmental stress, then a comparison between the two treatments is appropriate.

My results show that at the upstream sites (1, 6, 2), densities on both unseeded block and patty patch reefs were clearly below success criteria. This means that adding bare substrate to these sites did not appreciably increase the population size, and therefore these sites are not substrate limited. Mean densities on bare patch reefs at the two most downstream sites (4, 5) finished above success criteria, indicating that adding bare substrate did appreciably increase the populations on those sites. This suggests that substrate limitation is more important at these sites, and that an oyster population above success criteria could be potentially be established on these sites just by adding bare substrate. I classify the downstream sites as substrate limited, acknowledging that an oyster density of 15 oysters m^{-2} is not the quintessential mark of substrate limitation. Bare substrate deployed to high salinity, intertidal oyster leases for seeding prior to NREOH construction recruited oysters in densities equivalent to over 2000 oysters m^{-2} (Fig. 10). Substrate limitation at these locations is clearly much stronger than at any location in Farnell Bay. It does seem apparent though that while substrate limitation occurs on a continuum, there is a point down the

continuum where the population increase from added substrate becomes small enough that it is no longer meaningful. Using a metric of 15 oysters m^{-2}, that point occurs in Farnell Bay.

Larval preference for a particular substrate is a factor that may impact settlement, and thus in a substrate-limited population will influence population size and density (Connell 1985). Pediveliger oyster larvae can crawl over substrate during the beginning of the settlement process, exploring it for an ideal location to permanently attach. They will return to the plankton if they cannot find a suitable location. (Burke 1983). Marine epibenthos generally occur at a higher percent cover on heterogeneous substrate surfaces than on flat surfaces 1-3 months after settlement (Bourget et al. 1994), and oysters in particular prefer to settle on irregular surfaces (Galtsoff 1964). Given the gregarious nature of oyster settlement (Hidu 1969), it is logical to expect that higher densities of settlers will be found on preferred substrates.

Patch reefs made of the two different substrate material exhibited a similar number of oysters per patch reef at the end of the experiment. However, the block patch reefs contained a significantly higher surface area of substrate material than did the patty patch reefs. When compared using the metric of oysters per 0.1 m^2 of substrate material, the 2019 cohort recruited in higher densities to the patty material. A likely reason for this is larval preference for crevices and irregularities on the more rugous patties, although I cannot rule out that the chemical makeup of the material such as cement components and mixture played a role.

At upstream sites, post-settlement mortality of the 2019 cohort during the winter and spring of 2020 nearly erased the differences in densities between the two substrates. At downstream sites, differences between the two substrates remained in July 2020, and in some cases were enhanced. Thus, at the downstream sites, the patties appeared to facilitate post-settlement survival of newly recruited oysters better than the blocks. This study did not examine the mechanism for enhanced cohort survival on the patty substrate. One possibility is that the heterogeneity of the patty substrate provided refuge from predation. However, predation typically results in density dependent mortality in the prey population (Hixon and Carr 1997), while my results indicate that oyster mortality in Farnell Bay was density independent.

These results suggest that while patty material is superior for attracting oyster settlement, at upstream areas of high environmental stress this capability may not provide long term population enhancement. However, a superior substrate will maximize its effect on the population in areas of lower environmental stress where the enhancements to both settlement and post-settlement survival can impact the population over time.

All restoration sites met success criteria for the presence of reef material > 10 cm tall. (Fig. 17). This was an expected result, as the patch reefs were built to a height of 30-36 cm, but it is still consequential. Besides providing substrate for future oyster settlement on sites that previously had none, the presence of reef material increased nekton biodiversity on the sites (Owen Mulvey-McFerron, unpublished data). Additionally, based upon counts made while sampling oyster densities, I estimate that over 1,000,000 hooked mussels *Ischadium recurvum* recruited to the reef sites during the 18 months of the experiment. The co-occurrence of hooked mussels and oysters on reef habitat is not uncommon (Wells 1961). Assuming equal biomass between the two species, hooked mussels filter water at a rate 2/3 of that of oysters, while filtering picoplankton twice as efficiently (Gedan et al. 2014). Accounting for niche overlap between two important filter feeders during restoration planning will better account for ecosystem services enhanced by the restoration, and will provide increased justification for the restoration work (Coen and Luckenbach 2000, Gedan et al. 2014).

While oyster recruitment in Farnell Bay was very low in 2020, recruitment of the 2019 year class was strong enough at all six sites to meet criteria of evidence of recruitment in one out of two years (Fig 17). This held true for both patty and block patch reefs, although recruitment to a specified amount of surface area (0.1 m^2) was noticeably higher on the patty substrate (Fig. 15).

At the end of the experiment, with the exception of site 6, oyster density on the seeded patch reefs at all sites exceeded minimum success criteria of 15 oysters m^{-2}. At sites 3, 4, and 5, oyster densities exceeded target criteria of 50 oysters m^{-2}. At site 6, the margin of error overlapped minimum success criteria, so I cannot state with confidence that site 6 met success criteria for oyster density.

The largest divergence among sites in satisfying success criteria was on the two types of unseeded substrate. Lack of recruitment in 2020, and high mortality of the 2019 cohort during 2020 clearly yielded oyster densities below minimum success criteria at upstream sites (site 1, 6, 2). At downstream sites (sites 3, 5, and 4), margins of error either exceeded or overlapped minimum success criteria Fig 12).

It is important to note that I have made these declarations of restoration success 18 months after restoration. Restoration outcomes can follow many different trajectories over time, and can temporarily fluctuate above or below success criteria over short time scales (Bullock et al. 2011). Monitoring a restoration site for 1-3 years post restoration is useful for allowing managers to assess the immediate viability of the restoration. However, the system's response to restoration work may not become evident for decades (Borja et al. 2010). For this study, my assessment of successful oyster densities is driven primarily by the high densities of oysters on seeded patch reefs. These densities are declining (Fig. 11). It is possible that given strong year classes of recruits and sufficient intervals, followed by hospitable abiotic conditions, these densities could stabilize above success criteria over longer time scales. Given the evidence that I found for recruitment limitation, there is also a real possibility that densities will decline below success criteria over longer time scales.

This study indicates that recruitment limitation may be the most important factor effecting oyster populations in low salinity estuaries. Any oyster restoration attempts in an estuarine environment must consider the possibility that system is recruitment limited, and therefore must incorporate natural patterns of larval settlement and the abundance of potential recruits in its planning. The shell string data indicate that during this study, the restoration sites were located in areas of relatively low larval settlement. In a recruitment limited system, such populations will decline to densities dictated by settlement. This may be overcome by adding broodstock (Peterson et al. 1996), but restoration ecology does not yet have a precise formula to reliably overcome oyster recruitment shortfalls (Mann and Powell 2007). In Farnell Bay, we added a potential broodstock of at least 1 million oysters and found the system to still be recruitment limited. This may mean that a quantity of 1 million oysters was an insufficient restoration target.

However, several factors complicate prescribing a specific broodstock size to overcome recruitment limitation. If, as I assume, Farnell Bay is part of an open population whose larvae are sourced significantly from Stones Bay, adult population size and larval production in Farnell Bay are only one piece of the recruitment picture. Additionally, the broodstock that were added were all of the same year class. In protandric sequential hermaphrodites such as oysters, it may take several years and multiple year classes to achieve a distribution of male and female gametes in the population necessary for significant spawning (Galtsoff, 1964), although in warmer climates such as North Carolina and southward, some individuals will be able function as females during their first year of life (Coe 1936).

In the New River Estuary, I found that across 5 km of restoration sites in the same embayment, the magnitude of recruitment limitation and substrate limitation differed considerably between upstream and downstream sites. I also found that absence of evidence for recruitment limitation in a population depressed by environmental stress is quite different than absence of evidence for recruitment limitation in a high density population that experiences high settlement and is regulated by post-settlement mortality. The key driver of this differences is environmental stress, which in this study appeared to be low salinity or its covariate. Restoration managers must understand not only the abiotic environment in which they are working, but how specifically those abiotic factors will influence existing bottlenecks to their restoration attempts, such as recruitment limitation and substrate limitation.

A recruitment limited population will decline over time if losses from mortality are not replaced by recruitment. One way to maintain such a population is through regular, continued recruitment subsidies. Fish stocking programs are an example from natural resource management that are commonly practiced and commonly successful. The State of North Carolina has maintained stocks of striped bass *Morone saxatilis* in the Cape Fear, Neuse, and Tar-Pamlico Rivers since 1980, despite a lack of natural recruitment. This has been accomplished by stocking these rivers with 200,000 – 300,000 juvenile fish per year (Mathes et al 2020). While the stocking program has yet to result in sizeable, wild, reproducing striped bass populations, it does temporarily overcome recruitment limitation in these rivers, providing a valuable fishery as an ecosystem service that has lasted for the past 40 years and would not otherwise

exist. Likewise, provisioning of oyster reef ecosystem services could be maintained in recruitment limited portions of estuaries if stakeholders are willing to commit to subsidizing recruitment with a periodic oyster stocking program. Stakeholders would need to decide if the benefits of the ecosystem services are worth the expense of the subsidies, or if resources would be better applied in a system more suited to achieving a self-sustaining population. As is the case with most long-term manipulations of nature in order to produce specific services, managers should be vigilant lest their efforts gradually shift from advancing their understanding of the system and its natural processes in favor of attempts to increasingly control the system. Concurrently, society must be aware of the risks entailed if it becomes heavily dependent on a subsidized ecosystem service (Holling and Meffe 1996).

Although I investigated salinity as an abiotic driver of post-settlement mortality, I did not investigate biotic drivers of post-settlement mortality in either year. Oysters have many known predators, pests, and diseases whose impact varies by oyster life history stage and environmental conditions. Flatworms (Landers and Rhodes 1970), blue crabs (Eggleston 1990), mud crabs (Grabowski 2004), cow-nosed rays (Schwartz 1964), sheepshead (Macreadie et al. 2011) and black drum (Brown et al. 2008) are known predators of oysters and spat, and are present in Farnell Bay. A species of boring sponge *Cliona truitti* thrives in salinities similar to those common to Farnell Bay (Hopkins 1962), and throughout the experiment I observed boring sponge holes in oyster shells at the sites. However, this may not translate directly to an increase in predation mortality (Coleman 2014). Oyster tissue samples collected ad hoc in March 2020 revealed the presence of gonadal neoplasia and *Perkinsus marinus* infection in oysters at NREOH reef sites, (Tal Ben-Horin, personal communication) although I did not quantify the extent of the pathology in the population. *P. marinus* infection rates do not appear to increase in populations with high oyster densities, but conversely seem to be limited by high population densities and high recruitment (Bidegain et al. 2017).

TABLES

	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
Year	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
Mean	19.5	11.7	20.4	11.8	21.2	11.9	22.9	13.9	21.7	12.0	19.8	11.1
Min	5	0	7	0	8	0	11	3	9	3	4	2
Max	25	21	27	21	27	22	28	25	26	21	26	21

Table 1. Summary statistics for 2 years of refractometer salinities. Data consist of 20-23 point measurements per site per year.

	Site 1	**Site 4**	**Site 5**	**Site 6**
Mean	12.70	15.88	13.83	12.46
Median	12.74	16.31	14.29	12.86
Std. Dev.	2.43	2.29	2.51	2.46
Min	3.44	5.15	1.58	1.99
Max	17.04	20.93	19.25	18.10
% obs. < 10 psu	10	3	8	15

Table 2. Summary statistics from continuous salinity monitoring using HOBO U24-002-C conductivity loggers at four sites. Logging occurred from July – October 2020.

	October 2019		July 2020	
	Patty	Block	Patty	Block
Site 1	2.6 +/- 0.09	0.93 +/- 0.06	0	0.09 +/- 0.02
Site 2	1.5 +/- 0.21	0.85 +/- 0.12	0	0.11 +/- 0.01
Site 3	7.9 +/- 0.43	1.2 +/- 0.18	1.3 +/- 0.32	0.49 +/- 0.02
Site 4	23.7 +/- 7.4	2.1 +/- 0.16	5.8 +/- 2.4	0.47 +/- 0.05
Site 5	41 +/- 11	3.6 +/- 0.3	13 +/- 5.8	0.83 +/- 0.06
Site 6	12.3 +/- 2	0.46 +/- 0.05	0.61 +/- 0.43	0.25 +/- 0.05

Table 3. A comparison of mean densities on the two different types of substrate, per 0.1 m^2 of substrate material. Margin of error is given by the standard error of the mean. Samples were taken in October 2019 (n=2) to estimate the size of the 2019 cohort on each substrate, and again in July 2020 to assess the survival of the 2019 cohort nine months later.

FIGURES

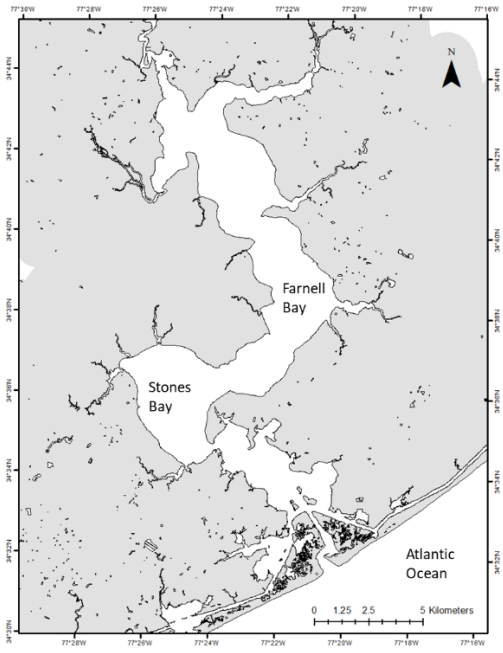


Figure 1. The New River Estuary, Onslow County, North Carolina.

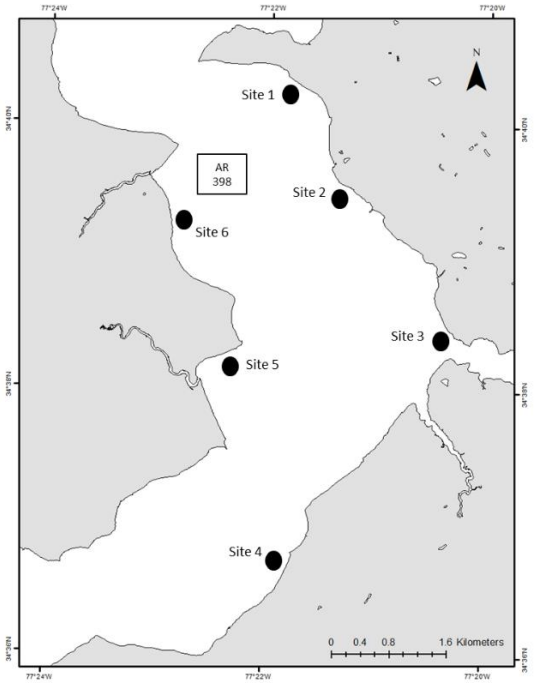
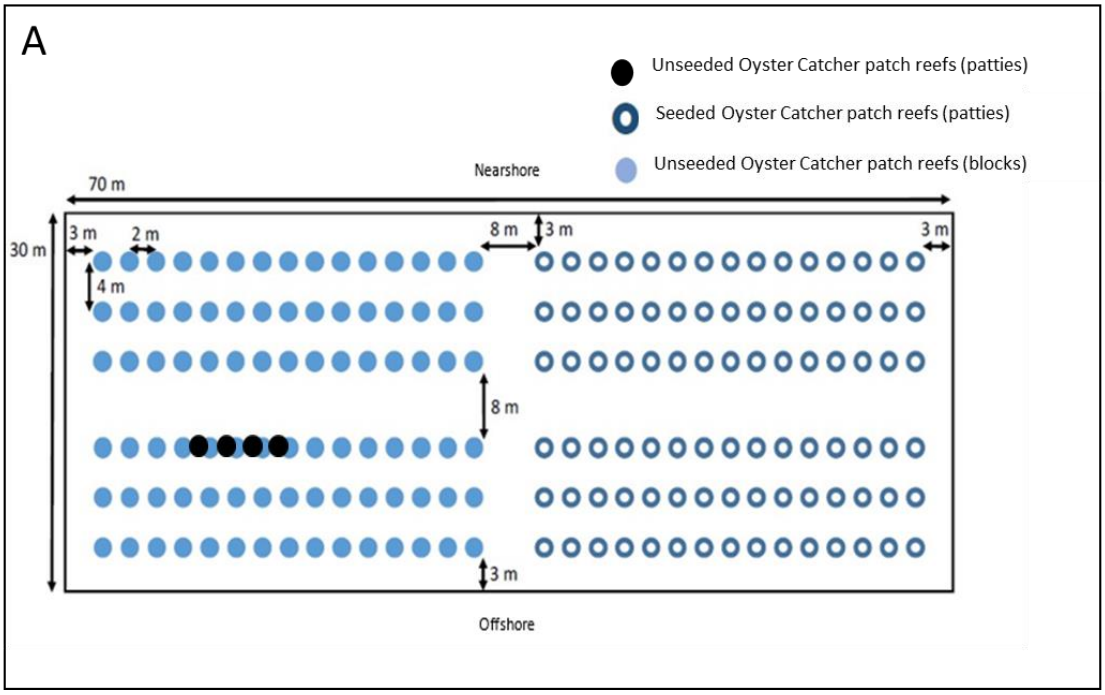
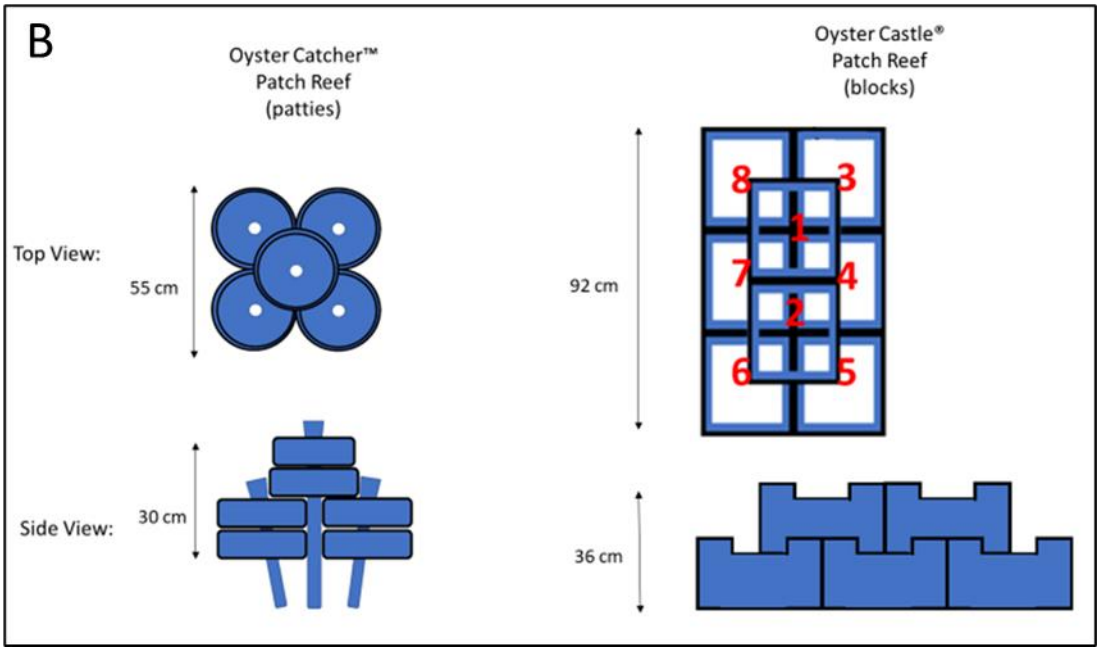


Figure 2. The New River Estuary Oyster Highway. The six restoration sites are spread across 5 km of Farnell Bay, in shallow water (<1m) within 100 m of shore, and reach to within 1 km of NC DMF Artificial Reef AR 398.


A
Unseeded Oyster Catcher patch reefs (patties)
Seeded Oyster Catcher patch reefs (patties)
Unseeded Oyster Catcher patch reefs (blocks)
Nearshore
70 m
30 m
3 m
2 m
8 m
3 m
3 m
4 m
8 m
3 m
Offshore


B
Oyster Catcher™
Patch Reef
(patties)
Oyster Castle®
Patch Reef
(blocks)
Top View:
55 cm
92 cm
8
3
1
7
4
2
6
5
Side View:
30 cm
36 cm

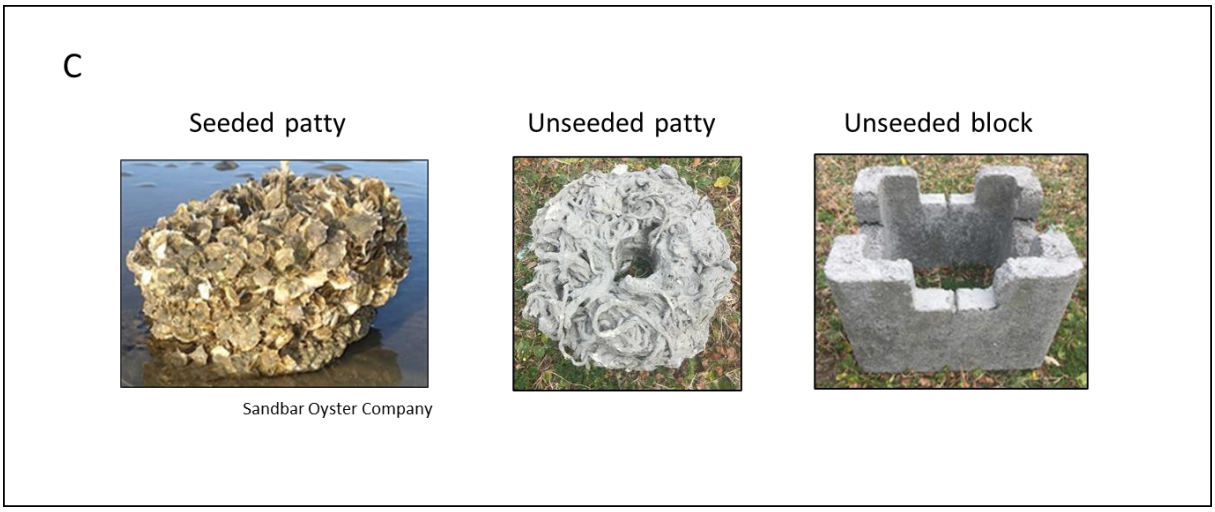


Figure 3. (a) Layout of a reef site, consisting of 90 seeded Oyster Catcher™ (patty) patch reefs, 90 unseeded Oyster Castle® (block) patch reefs, and four unseeded patty patch reefs. Construction occurred in April 2019, except for the four unseeded patty patch reefs added in June 2019. (b) Makeup of an individual patch reef. While the patch reefs are three-dimensional, oyster densities on one patch reef are a proxy for oyster density on 1 m^2 of seafloor. (c) Photographs of the individual pieces of substrate (patty or block) used to build the patch reefs.

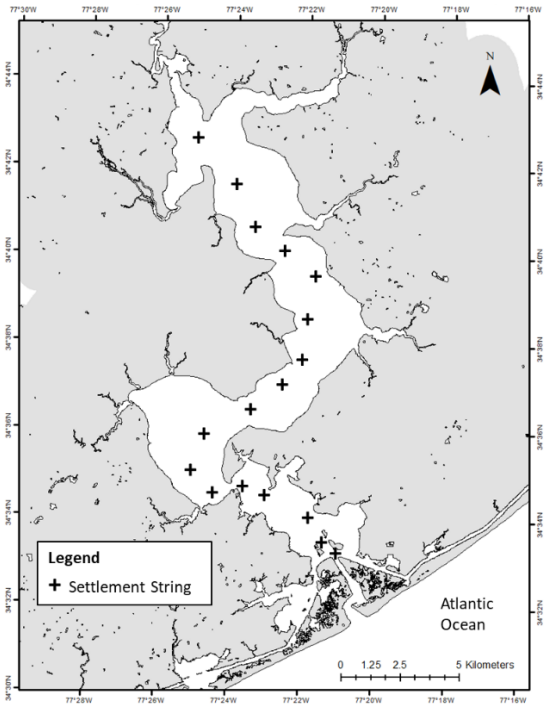


Figure 4. The 17 settlement string locations, spread across 25 km of the New River Estuary. Strings were hung along the main channel of the estuary from May to September during both 2019 and 2020, and checked every two weeks for evidence of oyster settlement.

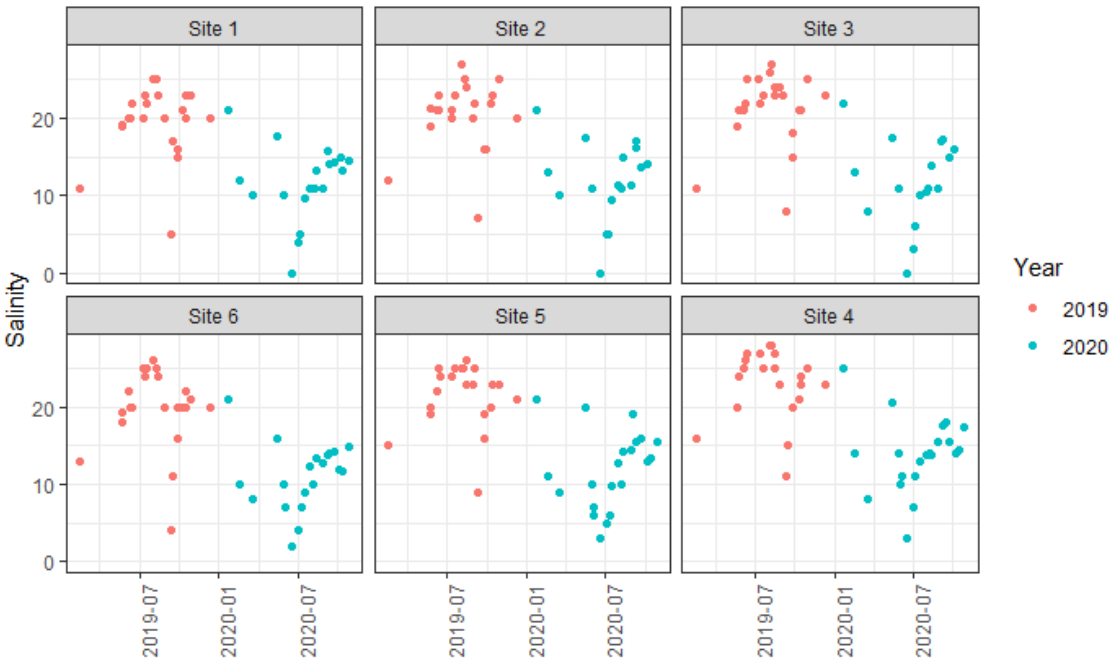


Figure 5. Farnell Bay salinity point measurements taken with a refractometer, 2019 – 2020. Data consist of 20-23 point measurements per site per year. The rapid decline in salinity in September 2019 occurred in the aftermath of Hurricane Dorian. There was also a freshet in June 2020 not associated with a named storm.

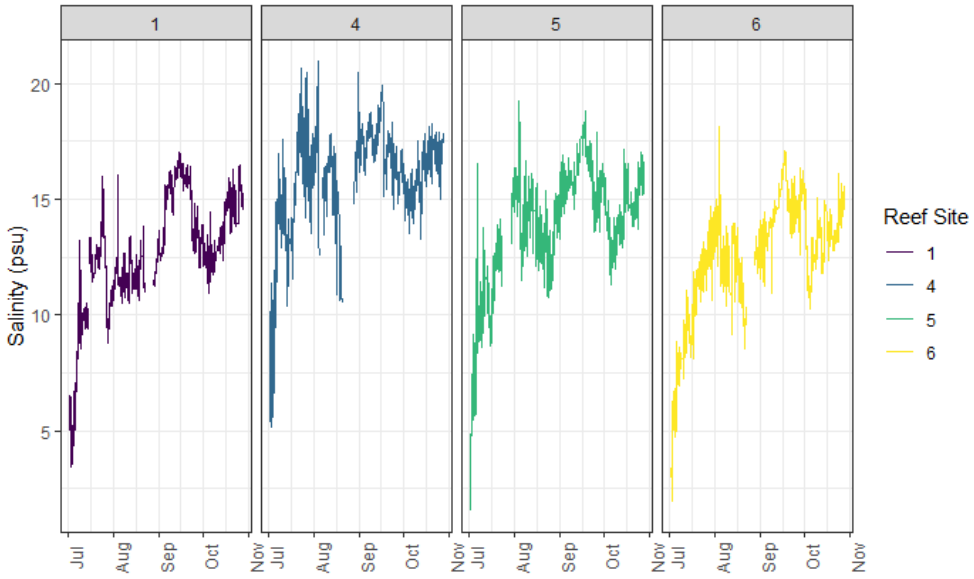


Figure 6. Continuous (every 2 hours) salinity measurements at 4 sites, July – October 2020. Logging began while salinities were very low from freshwater input the previous month.

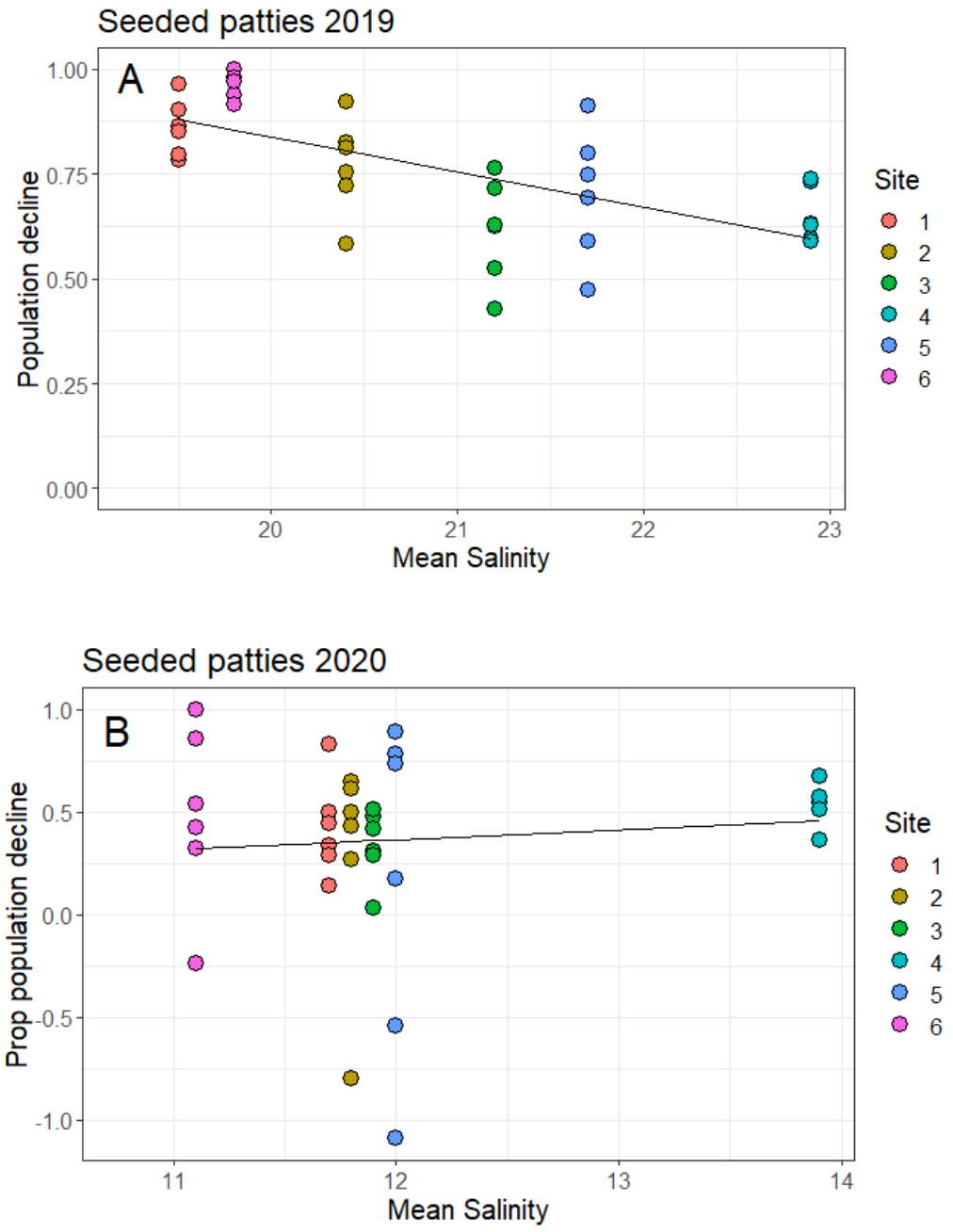


Figure 7. Proportional population decline (proxy for mortality) as a function of the mean of all refractometer salinity measurements taken that year, modeled with site as a random effect. (a) In 2019, populations on sites declined 8% per 1 psu decrease in salinity (p=0.054, 95% confidence intervals between 2% and 15%). (b) There was no correlation between salinity and population decline in 2020 (p=0.538, 95% confidence intervals overlapped zero)

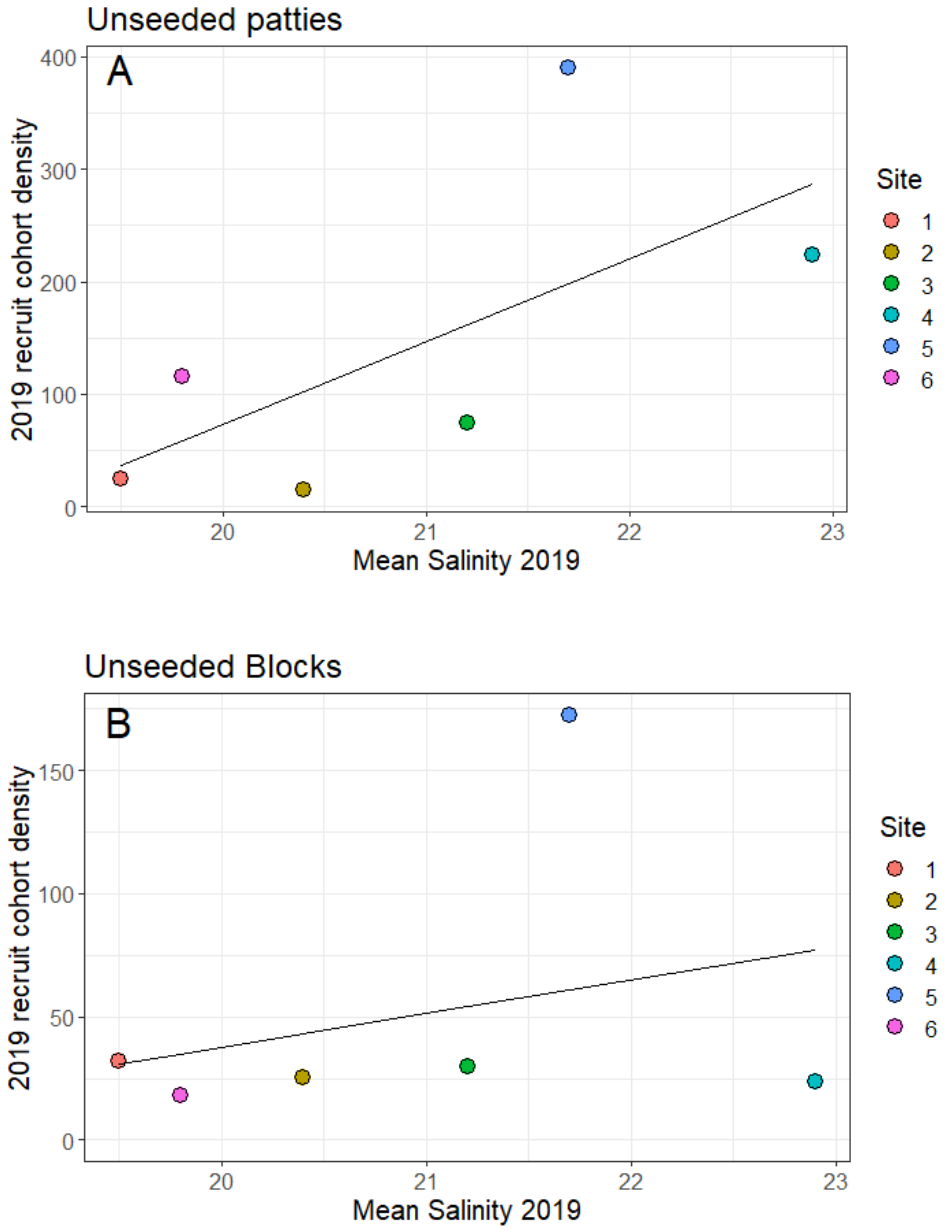
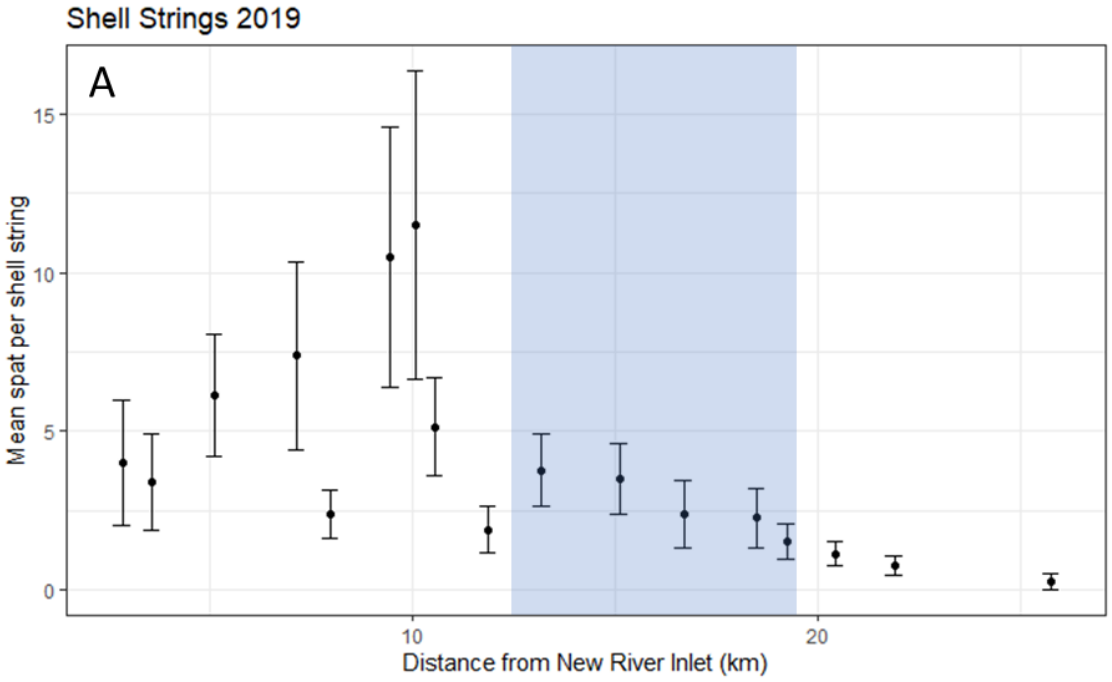
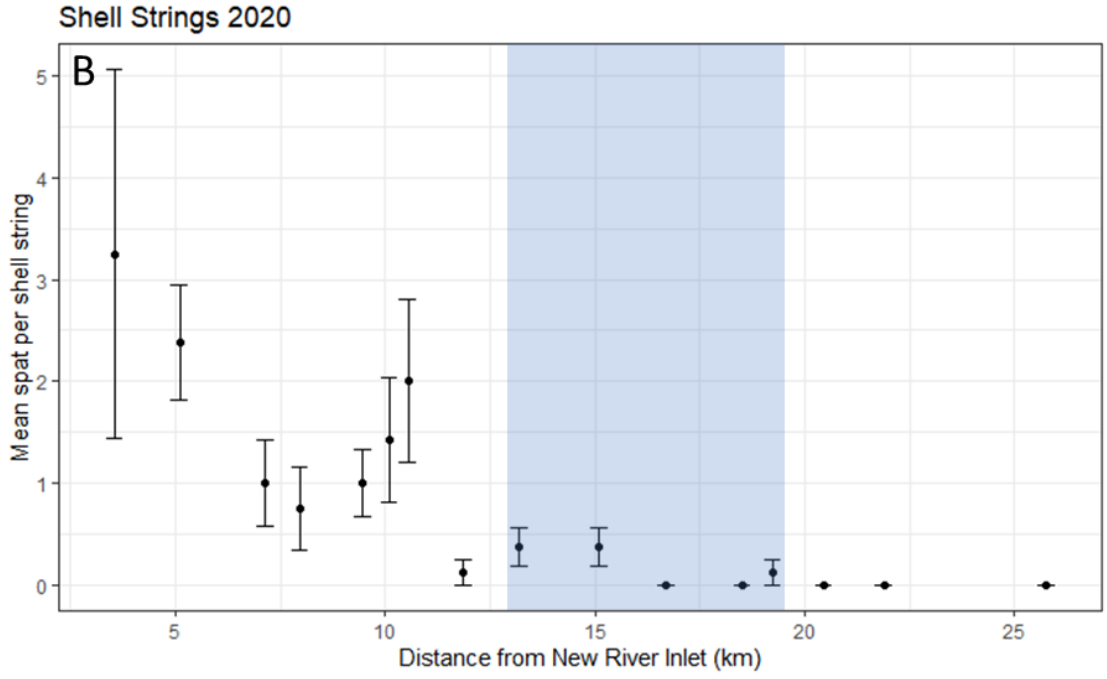
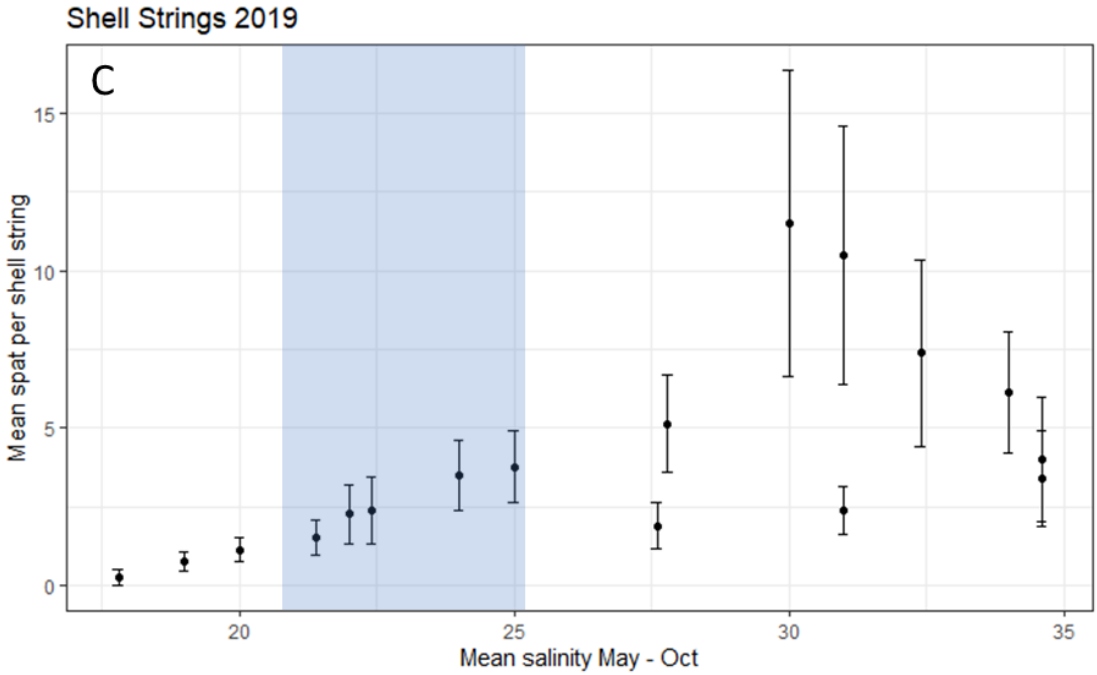
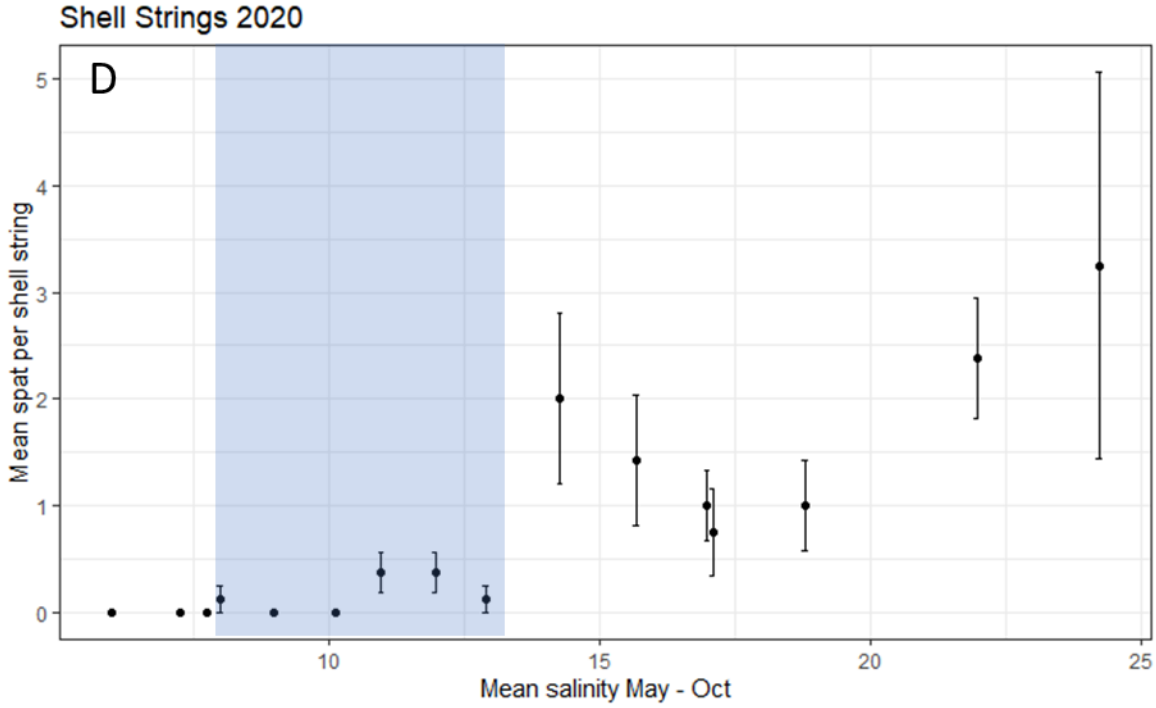


Figure 8. 2019 recruit cohort density as of October 2019 on unseeded patch reefs, as a function of mean salinity. No significant relationship was detected on either substrate type. (a) On unseeded patties, $p=0.161$, $R^2 = 0.27$. (b) On unseeded blocks $p=0.577$, $R^2 = 0$. Cohort density was highest at Site 5 on both substrate types.


Shell Strings 2019
A
Mean spat per shell string
0
5
10
15
10
20
Distance from New River Inlet (km)


Shell Strings 2020
B
Mean spat per shell string
0
1
2
3
4
5
5
10
15
20
25
Distance from New River Inlet (km)


Shell Strings 2019
C
Mean spat per shell string
0
5
10
15
20
25
30
35
Mean salinity May - Oct


Shell Strings 2020
D
Mean spat per shell string
0
1
2
3
4
5
10
15
20
25
Mean salinity May - Oct

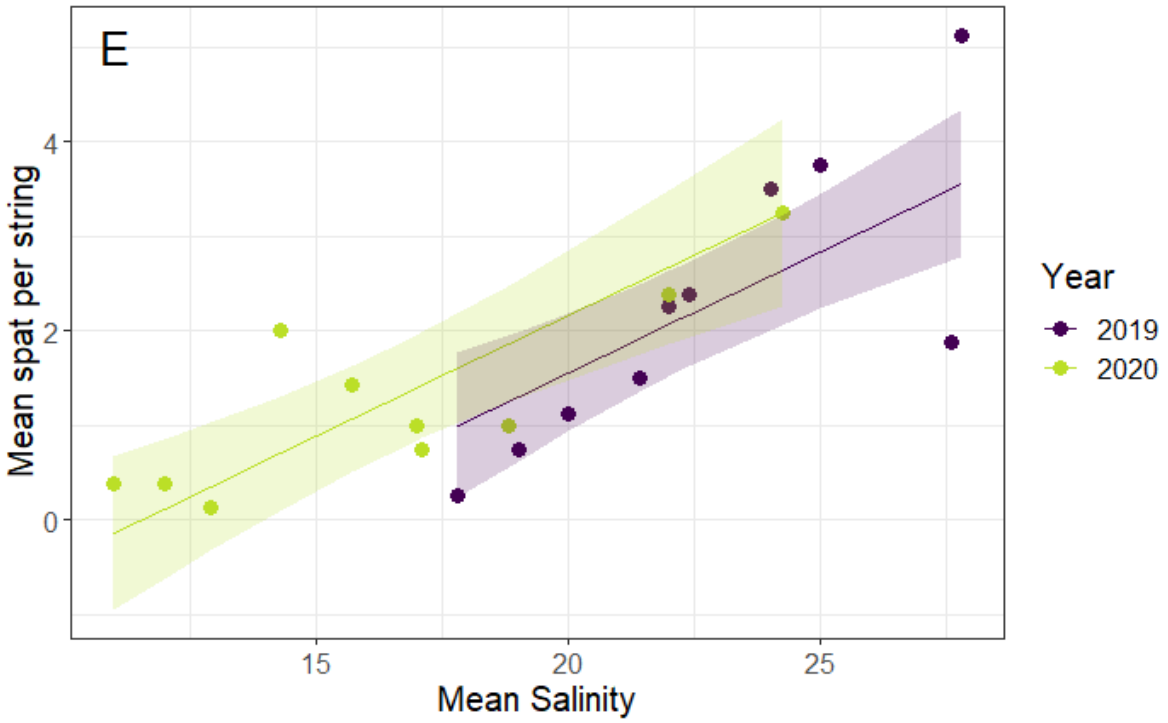


Figure 9. (a) Mean spat per settlement string plotted against distance from New River Inlet in 2019, and (b) against mean salinity at that string location in 2019. (c) and (d) show the same plots for 2020. The shaded area indications the portion of the estuary containing the NREOH reef sites. Highest recruitment in both years occurred at higher salinity locations downstream of Farnell Bay. (e) Mean spat per string as measured during the experiment both years, in the optimal oyster salinity window of 12-28 psu. Linear predictions modeled by ANCOVA are plotted, with 95% confidence intervals. Effect size was 0.26 spat per 1 psu, ($p < 0.001$ in 2019, $p = 0.007$ in 2020). $R^2 = 0.61$.

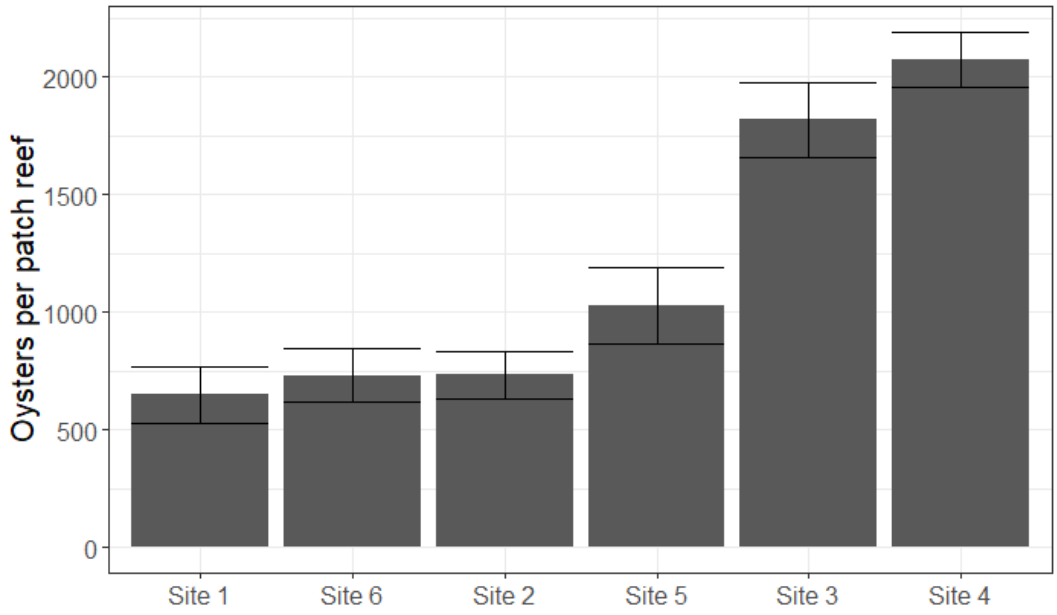


Figure 10. Initial densities of oysters on seeded patch reefs as measured in early June 2019. Although some mortality would have occurred between construction in April 2019 and June 2019, the plotted densities are indicative of the general differences between sites as determined by distribution of substrate during construction.

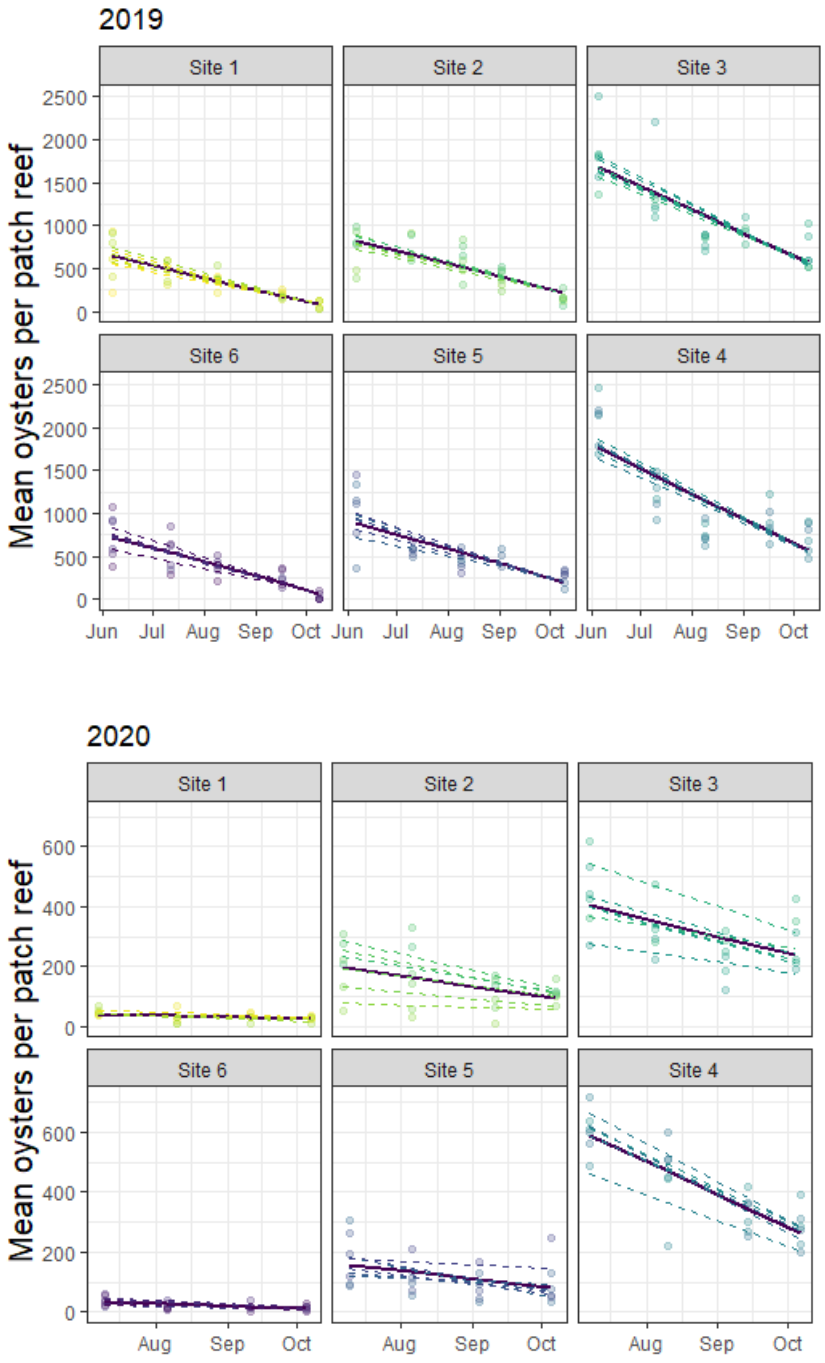


Figure 11. Population density decline in 2019 and 2020. Observed densities are represented by the points. Dashed lines depict model predicted mean for each patch reef (random effects). Solid lines indicate the model predicted mean for each site (fixed effects). Oyster density and rate of population decline was the steepest at Site 4 in both years. Rates of decline at Sites 1 and 6 in 2020 were not clearly different than zero.

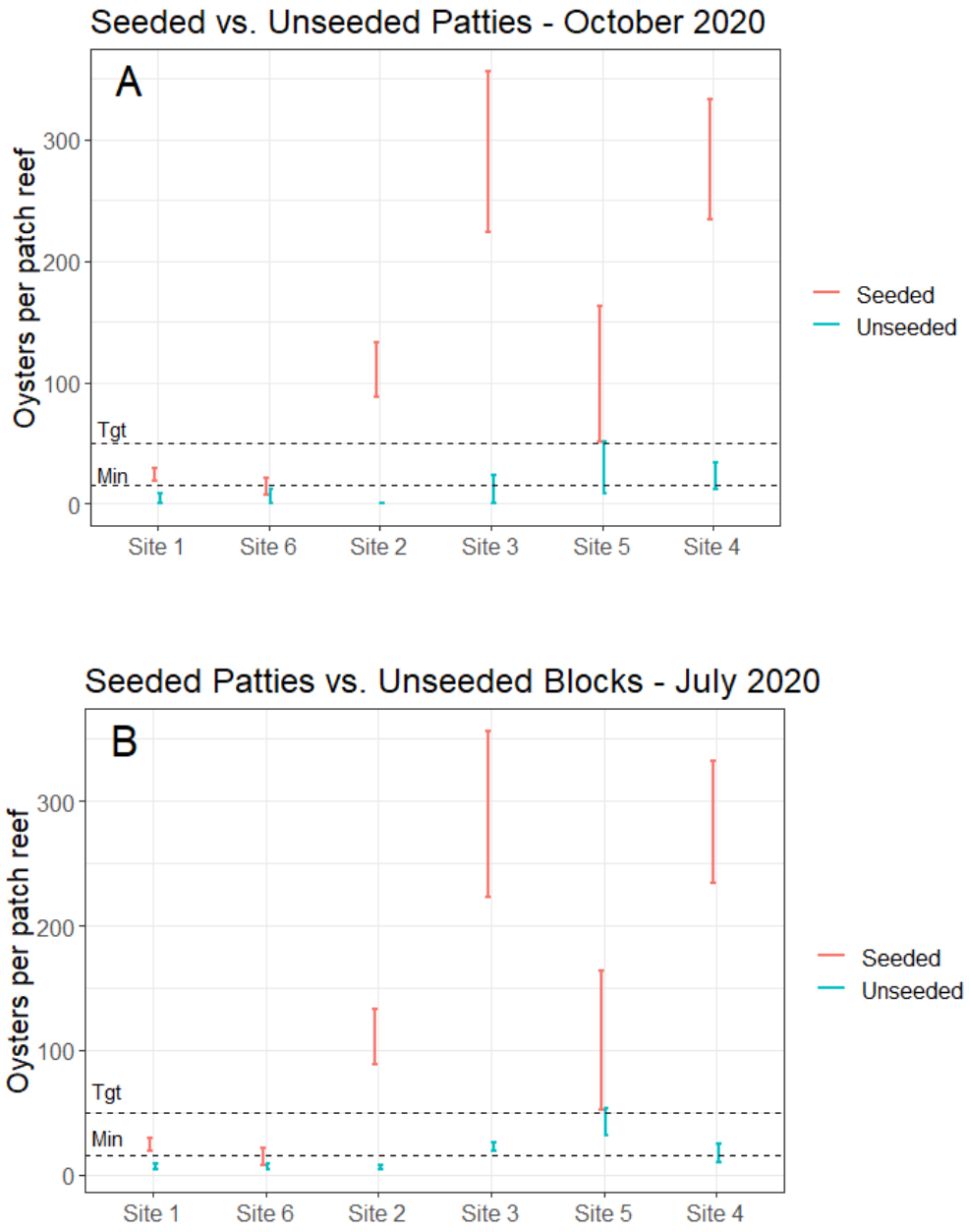


Figure 12. (a) A comparison of oyster densities on seeded and unseeded patty patch reefs in October 2012. Differences were greatest at upstream sites. At Site 6 there was no difference between seeded and unseeded patch reefs (p=0.269). (b) A comparison of oyster densities on seeded patty and unseeded block patch reefs in July 2020. Patterns are similar between both substrates. Horizontal dotted lines indicate minimum and target metrics of success.

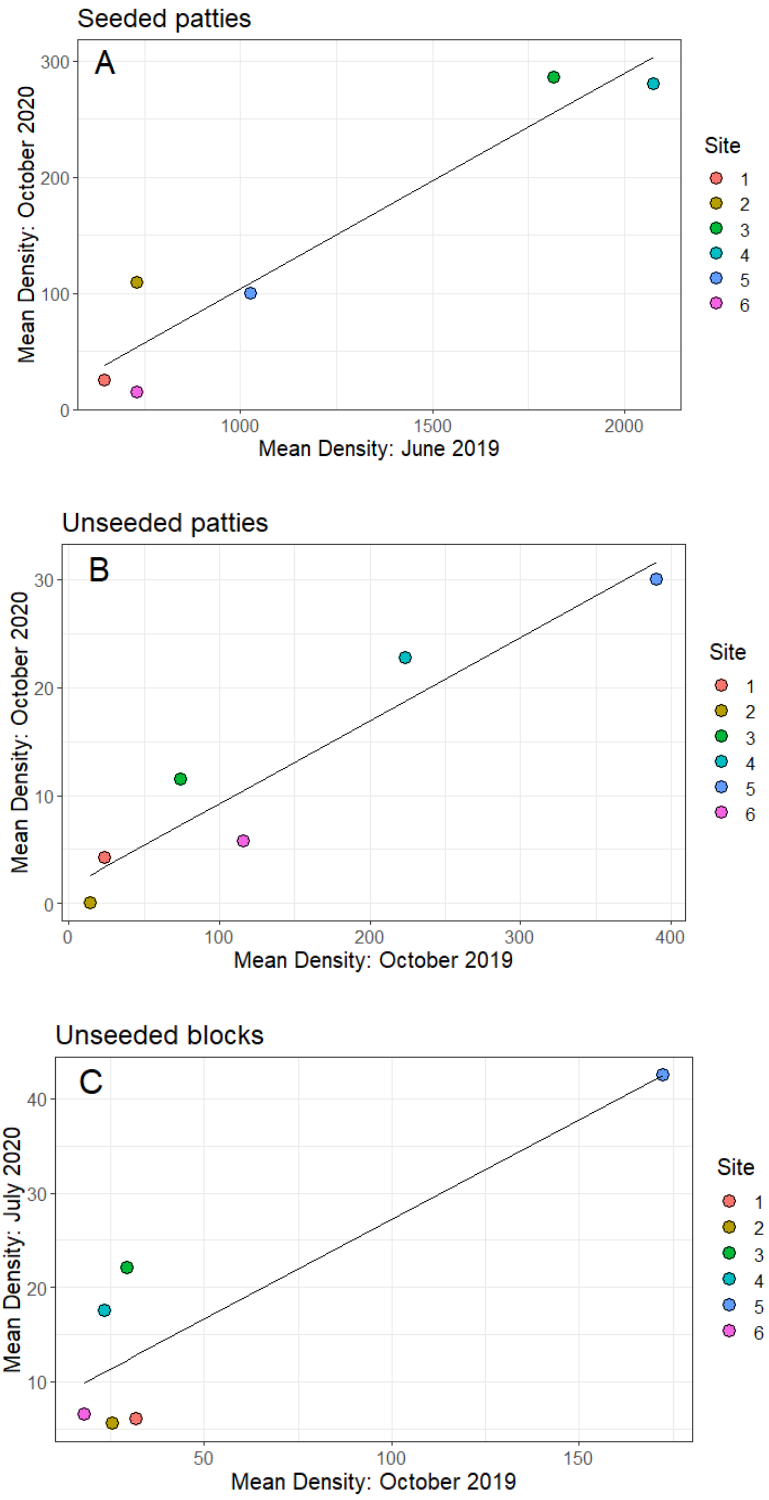


Figure 13. Mean oyster density at the end of the experiment as a function of mean oyster density at the beginning of the experiment on (a) seeded patty patch reefs ($p=0.003$, $R^2=0.89$), (b) unseeded patty patch reefs ($p<0.001$, $R^2 = 0.94$), and (c) unseeded block patch reefs ($p=0.02$, $R^2 = 0.72$). Correlation on unseeded blocks is less reliable due to the clustering of points near the origin.

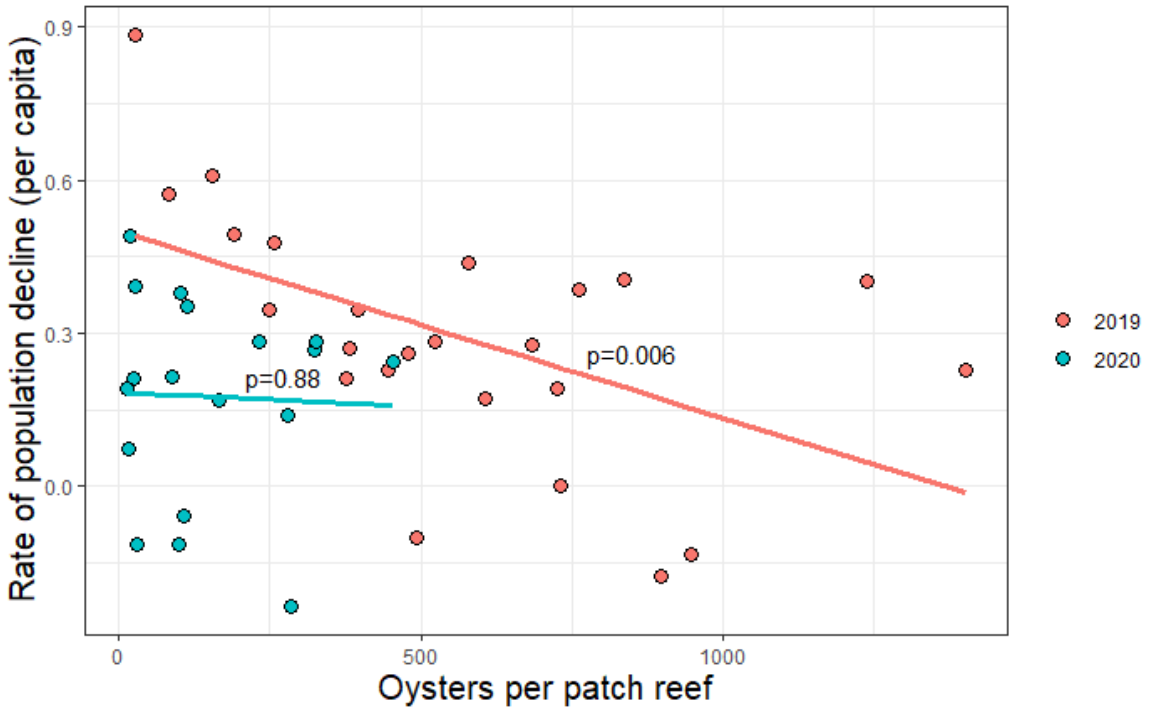


Figure 14. Per capita population decline on seeded patch reefs each month during sampling, as a function of the site's mean oyster density that month. This is a test of density dependent mortality, modeled with ANCOVA. The rate of population decline in 2019 appears to be negatively density dependent ($p = 0.006$). In 2020 there is no evidence of density dependence ($p=0.88$).

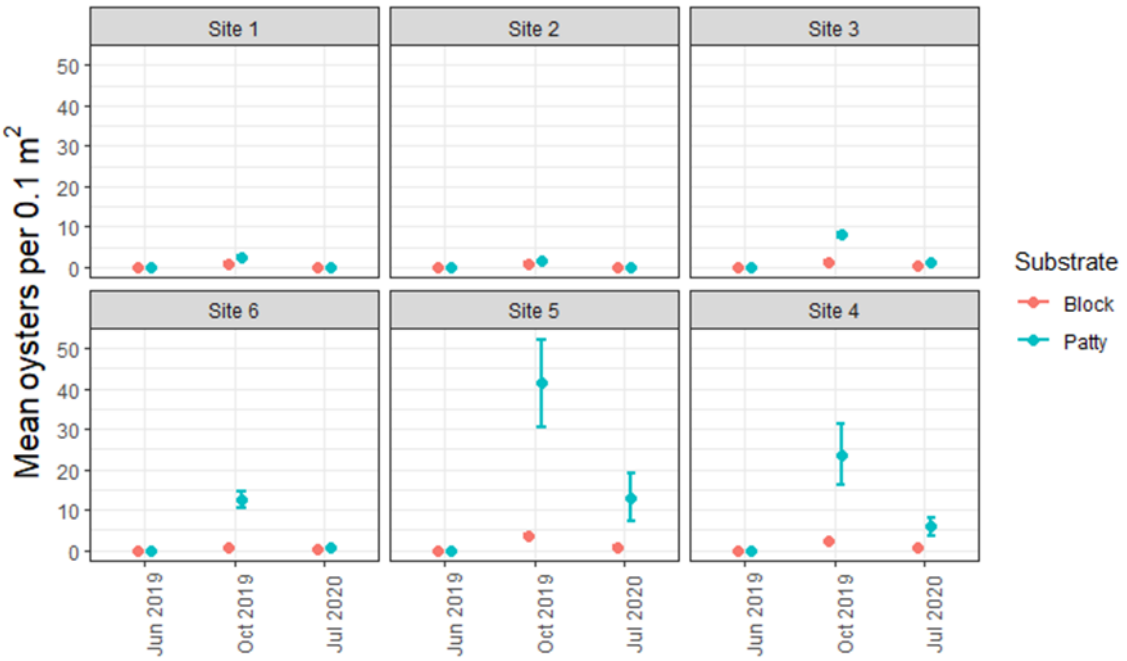


Figure 15. A comparison of densities on the two difference types of substrate. Densities were calculated per 0.1 m^2 surface of specific substrate, not seafloor. The densities on block substrate were adjusted to not account for substrate surface area lost to burial. Patty substrate saw enhanced recruitment compared to block substrate, particularly at sites receiving more overall settlement. Densities of the 2019 cohort declined from October 2019 to July 2020 on both substrates.

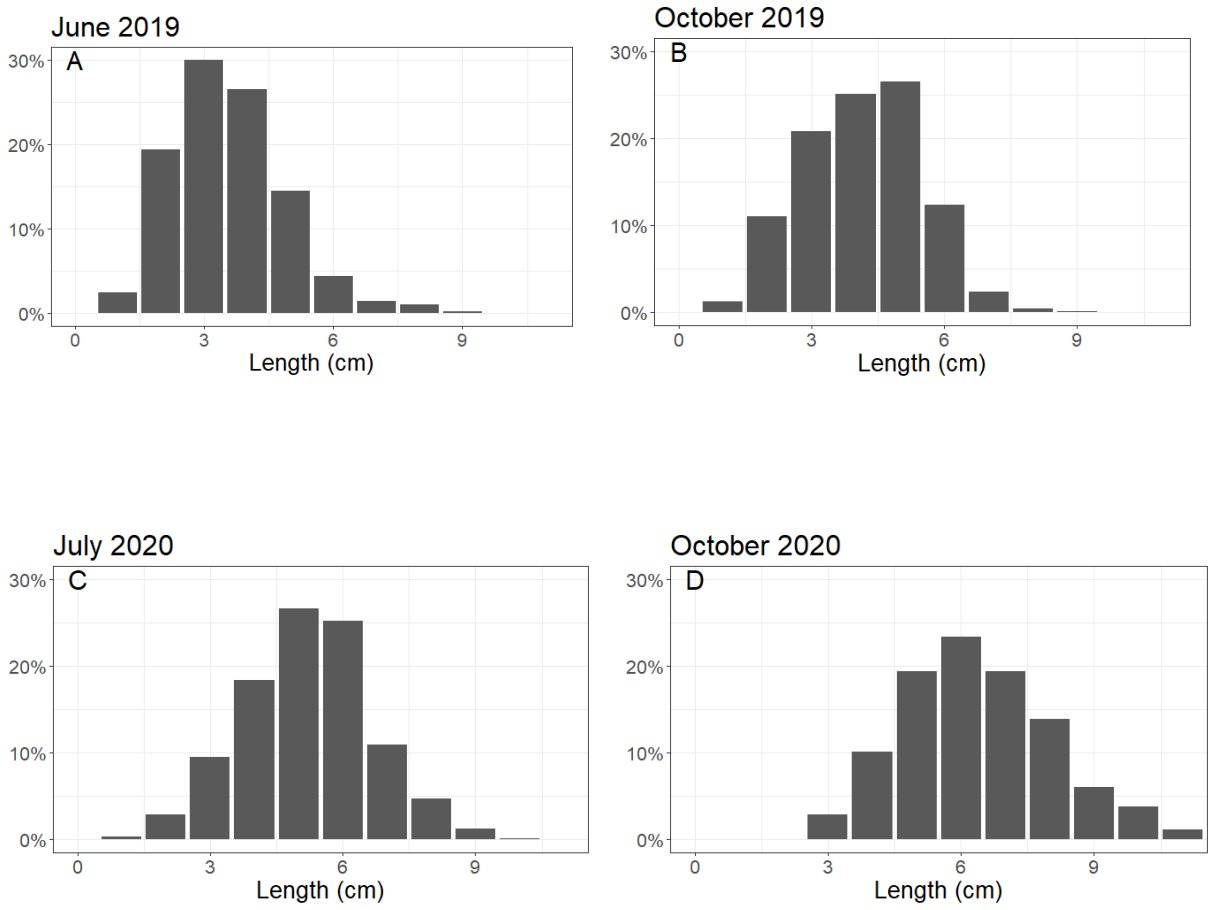


Figure 16. Frequency distributions of oyster lengths on seeded patty patch reefs over the course of the experiment. The mode increased from 3cm at the beginning of the experiment in June 2019, to 6 cm at the end of the experiment in October 2020.

	Presence of Reef Material	Evidence of Recruitment in 1 out of 2 Years		Density > 15 m-2 (Target > 50 m^{-2}) as of October 2020		
		Unseeded Patties	Unseeded Blocks	Seeded Patties	Unseeded Patties	Unseeded Blocks*
Site 1						
Site 6				?		
Site 2						
Site 3					?	
Site 5					?	
Site 4					?	?

Figure 17. Success criteria matrix at the end of the experiment in October 2020 (*density on block substrate was measured in July 2020). Green indicates success criteria met, red indicates success criteria not met. All sites met success criteria for presence of reef material and evidence of recruitment. All sites except Site 6 finished the experiment with some densities above success criteria, driven by densities on seeded patch reefs. The seeded density at Site 6 straddled the margin of error. Densities on unseeded patch reefs were clearly below success criteria at upstream sites (Sites 1, 6, 2) and either above success criteria or on the margin of error (indicated by gray box) at downstream sites (Sites 3,5,4).

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